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INTERNATIONAL STUDIES AND EVALATIONS IN THE FIELD OF

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EDITORS

PROF. DR. ENGİN ŞAHNA

PROF. DR. HASAN AKGÜL

PROF. DR. ZELİHA SELAMOĞLU

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e-mail: seruvenyayinevi@gmail.com

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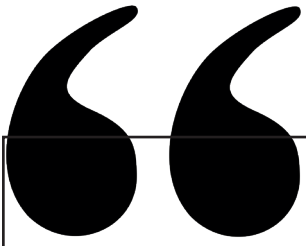

Chapter 1

EFFECT OF PROPRIOCEPTIVE NEUROMUSCULAR FACILITATION STRETCHING TECHNIQUE ON FUNCTIONAL STATUS IN CHILDREN WITH CEREBRAL PALSY

Emine Bilge ŞENLİK[1]

Selda BAŞAR[2]

Uğur SÖZLÜ[3]

Mustafa Necmi İLHAN[4]

1 Msc, Emine Bilge ŞENLİK. Sheffield Teaching Hospitals. NHS Foundation Trust Sheffield, UK. bbilgekisla@gmail.com ORCID No: 0000-0001-7172-5330

2 Prof. Dr. Selda BAŞAR. Department of Physical Therapy and Rehabilitation, Health Science Faculty, Gazi University Ankara, Turkey. seldabsr@yahoo.com ORCID No: 0000-0002-1433-4349

3 Dr. Lecturer. Uğur SÖZLÜ. Department of Physical Therapy and Rehabilitation, Health Science Faculty, Gaziosmanpasa University Tokat Turkey. sozluugur@gmail.com ORCID No: 0000-0001-5171-161X

4 Prof. Dr. Mustafa Necmi İLHAN. Department of Public Health, Gazi University School of Medicine, Ankara, Turkey. mnilhan@gazi.edu.tr ORCID No: 0000-0003-1367-6328

INTRODUCTION

Cerebral palsy is a non-progressive condition of the developing fetus or newborn brain that limits activity and impairs the development of mobility and posture (Berker & Yalçın, 2005). The brain injury is irreversible and cannot be reversed, however the effects can be mitigated. Most afflicted children suffer progressive musculoskeletal pathologies (Gulati & Sondhi, 2018). Cerebral palsy's motor deficiencies are frequently accompanied with problems of sensation, cognition, communication, perception and/or conduct, as well as a seizure condition (El-Shamy & rehabilitation, 2017; Krigger, 2006).

Most children with cerebral palsy suffer from progressive musculoskeletal issues (Krigger, 2006; Vitrikas, Dalton, & Breish, 2020; Wiart, Darrah, & Kembhavi, 2008). Cerebral palsy is diagnosed using three fundamental criteria: a loss of neuromotor control that impacts movement or posture, a static brain lesion, and brain damage during birth or within the first few years of life (Krigger, 2006; I. Novak, Hines, Goldsmith, & Barclay, 2012; I. J. J. o. c. n. Novak, 2014). Because of the scope of these criteria, cerebral palsy is an exceedingly variable diagnosis in terms of clinical presentation, etiology, and pathology (Krigger, 2006; Vitrikas et al., 2020). Although the brain lesions that cause cerebral palsy may not progress, the clinical picture may change over time as the affected individual grows and develops (Krigger, 2006; Tecklin, 2008; Vitrikas et al., 2020). Cerebral palsy can be classified based on a variety of criteria, including topography, mobility abnormality, and function. Cerebral palsy is classified into three kinds based on movement disorders: Cerebral palsy, dyskinetic, and ataxic (Gulati & Sondhi, 2018; Krigger, 2006). The present categorization excludes hypotonic-type cerebral palsy. The majority of infants who are hypotonic in early infancy develop cerebral palsy, which can be ataxic, dyskinetic, or both. However, some children may stay hypotonic due to the involvement of other systems (Gulati & Sondhi, 2018).

The prevalence of cerebral palsy, which is seen in childhood and is the most common cause of physical disability, is 2-3 in every 1000 live births in developed countries (I. Novak et al., 2012; I. J. J. o. c. n. Novak, 2014; Tecklin, 2008). Some affected babies may not survive, and the rate may vary from 1 to 5 in 1000 babies in different countries (Berker & Yalçın, 2005). The incidence of cerebral palsy increases significantly in multiple pregnancies. This rate is 15 per 1000 live births in twin pregnancies, while it can go up to 43 per 1000 live births in quadruplet pregnancies. In premature or very low birth weight babies, this rate is between 40 and 100 per 1000 live births (Bax et al., 2005; Gulati & Sondhi, 2018). A research done in Turkey found that the rate of cerebral palsy in children aged 2 to 16 was 4.4 per 1000 live births. The explanation for this high prevalence in our country is due to variables such as illnesses contracted during pregnancy and consanguineous marriage (Serdaroğlu, Cansu, Özkan, Tezcan, & neurology, 2006). The cause of cerebral

palsy is not completely understood (Krigger, 2006; Tecklin, 2008; Vitrikas et al., 2020). Potential reasons are investigated in a wide variety of categories, including perinatal, prenatal, and postnatal influences. The most prominent risk factors for cerebral palsy are low birth weight and preterm delivery. Decreased birth weight and gestational age increases the chance of cerebral palsy (Karaduman, Alemdaroğlu, & Yılmaz, 2014). The most frequent form is Cerebral Palsyastic cerebral palsy, and its incidence rate in all cerebral palsy is around 44% (El-Shamy & rehabilitation, 2017). It is commonly accompanied with periventricular leukomalacia and periventricular hemorrhagic infarction (Gulati & Sondhi, 2018).

One of the most prevalent consequences is hip contracture (Pinero et al., 2012). The Iliopsoas muscle is the primary source of this contracture (Bialik et al., 2009). Cerebral palsyasticity in this muscle impairs standing and walking ability by reducing extension movement in the last phase of stride (Bialik et al., 2009; Dostal & Andrews, 1981; Malai, Pichaiyongwongdee, & Sakulsriprasert, 2015). Stretching is the most frequent method for increasing range of motion, and it is an important part of both rehabilitation and exercise protocols (Holt, Baagøe, Lillelund, Magnusson, & neurology, 2000; Weppler et al., 2014). Proprioceptive Neuromuscular Stretching is a stretching technique used to increase muscle suppleness. It has been shown to have a positive effect on both active and passive range of motion (Hindle, Whitcomb, Briggs, & Hong, 2012; Lucas, Koslow, & skills, 1984; Szafraniec, Chromik, Poborska, & Kawczyński, 2018). It is characterized in the literature as the most effective stretching technique for short-term alterations, particularly when the goal is to enhance range of motion. There are various distinct proprioceptive neuromuscular facilitation stretching strategies, but the most frequent is Hold-Relax (Al Dajah, 2014; Feland, Myrer, & Merrill, 2001; Hindle et al., 2012). This procedure involves statically stretching a muscle, isometrically contracting it, and then statically stretching it again (Wicke, Gainey, Figueroa, & Research, 2014).

In a previous study, no difference was found in the development of balance and gait parameters in children with cerebral palsy who were included in a combined exercise program and static stretching applied to the hamstring muscle compared to the control group (Hindle et al., 2012). In contrast, Palmer et al. examined the effect of static stretching applied to the hamstring muscle on balance in healthy adult males and found a significant difference in the static stretching group compared to the control group (Palmer, Agu-Udemba, Palmer, & sportsmedicine, 2018). The aim of this study was to investigate the effect of proprioceptive neuromuscular facilitation stretching technique on functional status in children with cerebral palsy.

METHODS

Participants

This was a pilot study. This study was carried out at Gazi University Faculty of Health Sciences and Etimesgut Municipality Sacettin Gürbüz Barrier-Free Life, Cerebral Palsyecial Education, and Rehabilitation Center, with ethical permission from the University Faculty of Medicine Clinical Research Ethics Committee. Our study comprised seven Cerebral Palsyastic diparetic and triparetic children diagnosed with cerebral palsy. After informing parents and pediatric patients about the study, participants who signed the "Consent Form" were enrolled in the study.

The study's inclusion criteria were: having been diagnosed with cerebral palsy, being between the ages of 6 and 18, not having received Botulinum Toxin in the previous 6 months, having an IQ value of 70 or higher, having a GMFCS value of 1, 2, or 3, having hip flexion contracture, and agreeing to participate in the trial. Our study excluded patients with an additional diagnosis other than cerebral palsy and those who had undergone tendon lengthening surgery to promote hip flexion. Finally, 7 subjects took part in the study (Figure 1).

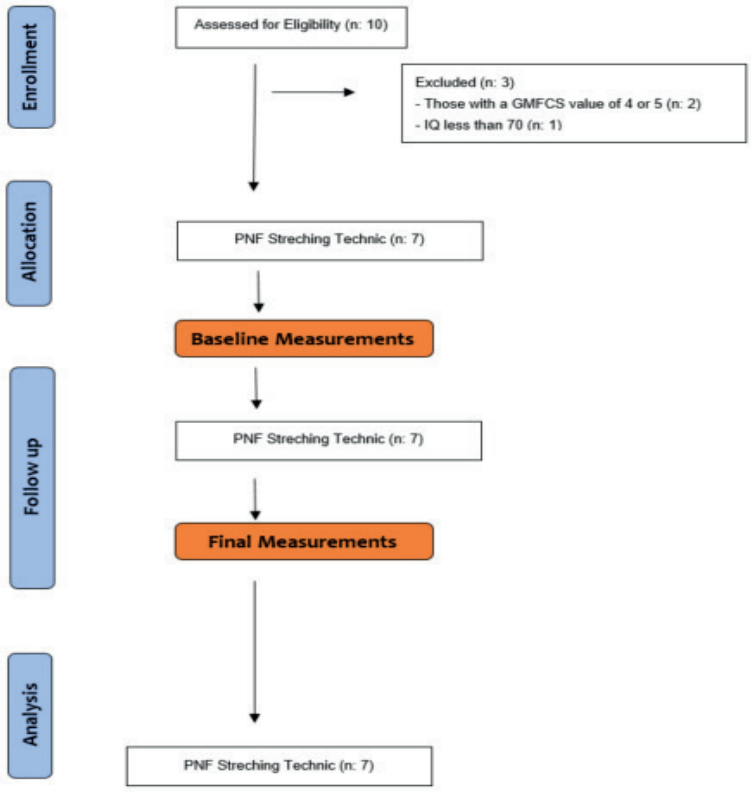

FIGURE 1. *Participant flowchart*

Study Design

Seven patients were included in this pilot study. Participants were given a program consisting of Proprioceptive Neuromuscular Facilitation stretching method exercises for four weeks, two days per week. During the proprioceptive neuromuscular facilitation stretching exercises, the children in the groups proceeded with their usual treatment program, which included strengthening, balance, and walking training. The following assessments were given to the participants before and after treatment.

Measurements

Demographic information

The children's demographic information (age, gender, cerebral palsy type, surgery history, height, weight, orthosis or devices used, IQ value, and GMFCS score) was collected prior to the study.

Time up and go test (TUG)

Balance and functional status were assessed using the timed up and go test (ICC: 0.83-0.89). The test began with the hip, knee, and ankle flexed at 90* while seated in a chair with no arm support. The patient was instructed to get up from the chair, walk the prescribed 3 m distance as quickly as possible, and then return to the chair with the command 'Start'. When the hips came into contact with the chair, the recording was halted, and the time was recorded in seconds. The test was completed in the shoes they normally used, and they were permitted to use walkers or crutches as needed. Those who utilized assistance gadgets were documented (Iatridou & Dionyssiotis, 2013).

1 minute walking test (1MWT)

In the 1-minute walking test to assess walking Cerebral Palsyeed, children were requested to stand up from an adjustable-height chair and walk a 20-meter-long oval shape as quickly as possible without running at the first order. When one minute had passed, the distance traveled in meters was measured using markers on the track. They were allowed to utilize walkers or crutches during the exam, depending on their needs. Those who used assistance gadgets were documented (McDowell, Kerr, Parkes, Cosgrove, & neurology, 2005).

Proprioceptive neuromuscular facilitation Stretching Technique

In addition to usual strengthening, balancing, and walking activities, the children were taught the proprioceptive neuromuscular facilitation stretching technique, which was administered to the hip flexors on both sides for four weeks, two days a week (Al Dajah, 2014; Feland et al., 2001; Hindle et al., 2012). There was no set order for which side to begin the procedure. For this approach, the patient was supine. While the hip and knee joints on the non-stretching side were kept at 900 flexion, the opposite thigh was extended

from the hip, leaving it out of the bed as far as the range of motion permitted. Stretching was done for 20 seconds from immediately proximal to the patella in the extension direction until the Visual Analog Scale indicated a sense of moderate (4-6) stretching at the conclusion of the extension. The individual was next requested to draw his knee towards his belly while performing hip flexion, with resistance provided just proximal to the patella, for 20 seconds of isometric hip flexion. The power exerted during isometric hip flexion was measured in pounds using a portable dynamometer (K-Forca Muscle Control Muscle Strength Measuring Device) (Malai et al., 2015) (Figure 2). Following this application, the patient was advised to rest before receiving another 20-second extension stretch. The technique was repeated six times. Each cycle was separated by a 20-second rest period (Aslan, Buddhadev, Suprak, & San Juan, 2018). In addition, the patient was given a 2-minute rest period before repeating the procedure on the opposite side.

Figure 2. *Proprioceptive neuromuscular facilitation stretching technique*

Data Analysis

Statistical analysis was carried out with the Windows-based SPSS 22.0 statistical analysis program. Variable conformance to the normal distribution was determined using both visual (histograms and probability graphs) and analytical approaches (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed using mean and standard deviation for regularly

distributed data, percentages (%) for counting variables, and median and interquartile range (IQR) for non-normally distributed variables. The Wilcoxon Test was used to evaluate the groups before and after treatment when the data was not normally distributed. The data was reviewed at a 95% confidence level with a p-value of less than 0.05.

RESULTS

Characteristics of the subject

The demographic features of the groups are shown in Table 1. The ages of the children who underwent proprioceptive neuromuscular facilitation stretching were 12 (10-16) years, their heights were 150 (140-160) cm, and their body weights ranged from 47 (32-61) kg to 21 (16.5-24) kg/m^2.

Table 1. *Demographic characteristics of individuals*

	PNF stretching (n:7)	
	Median (IQR)	**X±SD**
Age (years)	12 (10-16)	13±3
Height (cm)	150 (140-160)	149±21
Body weight (kg)	47 (32-61)	47±17
Body mass index (kg/m^2)	21 (16.5-24)	21±4

Functional parameters

Functional test results of the individuals before and after proprioceptive neuromuscular facilitation stretching treatment are given in Table 2. Functional values (Time up and go test-1 minute walk test) were statistically similar (p>0.05).

Table 2. *Comparison of pre- and post-stretching functionality test results*

	PNF stretching (n:7)		p
	Median (IQR)	**Median (IQR)**	
	Before PNF stretching	**After PNF stretching**	
Time up and go test	13(11-16,2)	12,5(7-14.5)	0,325
1 minute walk test	52(33.5-74.6)	56(37-81.2)	0.483

DISCUSSION

The aim of this study was to compare the effectiveness of the proprioceptive neuromuscular facilitation stretching technique on functionality in children with cerebral palsy and hip contracture. As a result of our study, no significant

difference was found in the effects of the proprioceptive neuromuscular facilitation stretching technique on functional status.

Proprioceptive neuromuscular facilitation stretching is effective in increasing rom in children with cerebral palsy (Lucas et al., 1984). Additionally, proprioceptive neuromuscular facilitation stretching increases active and passive flexibility (Sharma & Cleland, 2016). The most commonly used method among proprioceptive neuromuscular facilitation stretching techniques is Hold-Relax (Yıldırım, Ozyurek, Tosun, Uzer, & Gelecek, 2016). There are studies in the literature showing an increase in hamstring flexibility and hip flexion range after the Hold-Relax stretching technique. (Spernoga, Uhl, Arnold, & Gansneder, 2001) (Bonnar, Deivert, Gould, & fitness, 2004). Theoretically, proprioceptive neuromuscular facilitation stretching is expected to stimulate not only muscle fibers but also sensory receptors in agonist and antagonist muscles (Lim, Nam, & Jung, 2014). This increase is expected to improve functionality by positively affecting balance and coordination.

Fosdahl et al. found no difference in the improvement in balance and gait parameters in children with cerebral palsy who were followed with a hamstring stretching and combined exercise program compared to the control group (Fosdahl, Jahnsen, Kvalheim, & Holm, 2019). In another study looking at the acute effect of proprioceptive neuromuscular facilitation stretching on balance, proprioceptive neuromuscular facilitation stretching was applied to the hip abductor and adductor muscles of participants and a significant difference was shown in the balance of these participants afterwards (Szafraniec et al., 2018).

This study's main limitation is its relatively small sample size, which was a pilot study. The study's findings should be validated in a multicenter randomized controlled trial with a larger sample size and blinded main outcome evaluation. DeCerebral Palsyite the small number of participants in this preliminary study, it provides important information that will assist influence the design and implementation of future clinical investigations.

CONCLUSION

Although proprioceptive neuromuscular facilitation stretching is thought to be more successful than other forms of stretching in increasing functioning, our study demonstrated no significant change in functionality among children who performed proprioceptive neuromuscular facilitation stretching. In the future, randomized controlled trials with a bigger number of patients and a control group will be advantageous for contributing to the literature.

Conflict of Interest and source of funding statement

The authors declare that they have no conflict of interest. This work has been supported by GAZİ University Scientific Research Projects Coordination Unit (under grant number: 47/2020-09).

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Chapter 2

THE EFFECT OF FDA-APPROVED DRUGS USED IN ALS TREATMENT ON GENES INVOLVED IN ALS PATHOGENESIS

Aslı Aykaç[1]

Ahmet Özer Şehirli[2]

1 Prof. Dr. Aslı AYKAÇ, Yakın Doğu Üniversitesi, Biyofizik AD
2 Prof. Dr. Ahmet Özer ŞEHİRLİ, Yakın Doğu Üniversitesi, Diş Hekimliği Fakültesi, Farmakoloji AD

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) resulting in eyebrow paralysis is a progressive, devastating, and lethal neurodegenerative disorder. ALS causes cognitive impairment as well as selective functional impairment in motor neurons (Feldman, 2022). The average longevity of ALS patients is 3-5 years, with barely 5 % of patients surviving 20 years (Mehta et al., 2022). Because of the aging of the world population, it is predicted that the number of people with ALS will increase by 69 % by 2040 (Arthur et al., 2022).

ALS appears to be a spectrum disorder rather than a single disease. While the age of onset of the disease in ALS patients is uncertain, many factors, such as the symptoms of the disease, the type of involvement, and the patient's survival, vary from patient to patient. ALS is a highly complex disease with molecular heterogeneity. Due to the similarity of the clinical signs seen in ALS to other neuromuscular and neurological diseases, the symptoms also make the symptoms very heterogeneous, which makes the diagnosis of the disease difficult. Cognitive and behavioral abnormalities (frontotemporal dementia: FTD) are also seen in ALS patients. ALS and FTD share genetic mutations and have a pathogenic interaction with one another (Wang et al., 2023). About 5–10 % of ALS patients are known as "familial ALS: FALS", while the rest are known as "sporadic ALS" (SALS). FALS is inherited in three different ways: autosomal dominant, autosomal recessive, or X-linked. In autosomal dominant inheritance, there is a 50 % chance that the child will develop FALS; in autosomal recessive inheritance, there is a 25 % chance that the disease will occur in the child. In X-linked inheritance, girls who inherit the mutated gene on the X chromosome from their father can pass on the disease as carriers, while boys who inherit the gene from an affected mother will be more prone to the disease. Approximately 70 % of cases in which ALS genes such as "superoxide dismutase 1: SOD1, fused in sarcoma: FUS, Transactive response DNA-binding protein: TARDBP, chromosome 9 open reading frame 72: C9ORF72, and Ataxin-2: ATXN2 are mutated belong to FALS and 15 % to SALS (Wang et al., 2023). Although the ALS phenotype may vary according to the gene region affected by the mutation in the hypothalamic-pituitary-adrenal (HPA) axis, there is no clear genotype-phenotype correlation in ALS because there are differences even in cases with the same mutation within the family Table 1.

The proper balance of protein production and degradation is crucial for cellular health. The accumulation of protein aggregates and damaged proteins due to oxidative stress reflects key characteristics of an aging cell. This phenomenon is particularly relevant in age-related neurodegenerative disorders, and there is extensive evidence for the involvement of proteostasis in the pathophysiology of ALS. Disruption of proteostatic mechanisms, as seen in many neurodegenerative diseases, including ALS, leads to the

accumulation of misfolded proteins, creating a vicious cycle that impairs cellular processes. The ALS pathomechanism involves RNA-binding proteins, ALS-associated genes, and non-coding RNAs. Reactive oxygen species (ROS) can cause neuronal damage by altering the structure and function of biological molecules such as proteins and RNA. The role of oxidative damage in ALS pathophysiology is well established. Oxidative stress disrupts RNA binding by acetylated TDP-43, leading to the accumulation of hyperphosphorylated TDP-43 species. In cellular experiments related to ALS, exposure to oxidative stress has been observed to result in mislocalization of proteins like TDP-43 and FUS, leading to RNA processing defects. Cytoplasmic aggregation of TDP-43 sequesters proteins, including mitochondrial proteins, and disrupts mitochondrial function. This further exacerbates oxidative stress, leading to a vicious cycle between oxidative stress, protein aggregation, and mitochondrial dysfunction. Another mechanism involved in the pathophysiology of ALS is mitochondrial dysfunction. Altered mitochondrial structure and function lead to disruptions in mitochondrial axonal transport and trigger various effects, including apoptotic mechanisms (Saxena et al., 2011; Wang et al., 2023). The processes involved in the pathomechanism of ALS and the genes involved in these processes are summarized in Table 2 and Figure 1. Variants in more than 40 genes have been identified to cause ALS, raise the risk of ALS, or be related to a variation in clinical phenotype since the late 1990s (Saxena & Caroni, 2011; Sweeney et sl., 2017).

ALS RELATED GENES

SOD1

The first ALS gene identified, SOD1, encodes a Cu/Zn-binding SOD. It has been suggested that ALS is a conformational disease, as SOD1 mutations reduce the enzyme's catalytic activity (less than 50 %). Mutation in the SOD1 gene, transactive response (TAR)-DNA binding 43 kDa (TDB-43), C9ORF72, and FUS can be counted among the main causes of FALS. More than 100 genes are reported to be associated with ALS, and mutations in the SOD1, C9ORF72, FUS and TARDBP genes are the most common genetic causes (Hardiman et al., 2017). There are 36 SOD1 variations, 11 FUS variants, and 14 TARDBP variants linked to ALS. While these mutations account for 60 % of FALS and 10 % of SALS, the impact of the remaining variants on cases is unknown (Yun & Ha, 2020).

TARDBP

The TARDBP gene encodes TDP-43, a DNA/RNA binding protein that functions on a variety of RNAs. TDP-43 regulates and controls various steps of RNA metabolism, such as RNA transport, translation, mRNA splicing, mRNA stability, pre-mRNA splicing, and microRNA synthesis (Lattante et al., 2013; Wang et al., 2023). TDP-43 is normally found in the nucleus, but in

pathological situations, the cleaved form is mainly found in the cytoplasm. Mutations in the TARDPB gene have been identified in approximately 3 % of FALS patients and 1.5 % of SALS patients, suggesting their role in a subset of ALS cases (Lattante et al., 2013). TDP-43 aggregates are identified in damaged areas of the cortex, brainstem, and spinal cord in ALS. TDP-43 is depleted from the nucleus in more than 95 % of the cells of ALS patients (Wang et al., 2023). The longer half-life of the mutant protein than the wild type is associated with accelerated onset of the disease. Although the onset of the disease is earlier in ALS patients carrying TDP-43 mutations, they rarely show a tendency to dementia. TDP-43 pathology is seen in 50 % of FTD patients and almost all ALS-FTD spectrum cases. TDP-43 pathology has been detected in non-motor regions of the brain of ALS patients who do not have dementia but exhibit cognitive impairments (Gregory et al., 2020). Some models have been proposed to explain the mechanism underlying TDP-43 toxicity. First, ALS-associated mutations increase cytoplasmic TDP-43 accumulation (loss of nuclear function) but also decrease its propensity to aggregate (gain of cytoplasmic function). Second, the formation of stress granules increases TDP-43 accumulation and aggregation. TDP-43 is widely expressed and is found primarily in the nucleus. It is also found in cytoplasmic RNA granules, axons, dendrites, and synaptic sites (Gelon et al., 2022). The fact that the affected gene products are mostly mitochondrial proteins leads to mitochondrial imbalance due to increased oxidative stress (Wang et al., 2023).

FUS

FUS is a type of protein similar in structure to TDP-43 that is involved in the metabolism of RNA. Most FUS mutations causing ALS are heterozygous with an autosomal dominant inheritance pattern. DNA damage repair and transcription are among the known functions of FUS. FUS protein is found in different locations outside the nucleus at the neuromuscular junction; this indicates that although it belongs to the nucleus, it has a unique role at the neuromuscular junction. FUS protein may function as a transcription factor that regulates the transcription of specific genes. In this approach, it actively regulates the transcription of cholinergic receptors. A deficiency or abnormal function of this protein can compromise the structural integrity of the neuromuscular junction and result in decreased nerve conduction. FUS protein contributes to the protection of the neuromuscular junction by regulating the formation and transport of ribonucleoprotein complexes in nerve cells. Although it is known that mutant FUS confers a toxic function to the cytoplasm through cytoplasmic mis-localization, its effect on neurodegeneration is not fully understood. It has been reported that the mislocalization caused by mutant FUS affects energy metabolism by increasing enzymatic interactions in glucose metabolism, which may play a role in FUS-associated ALS (Yun & Ha, 2020)

FDA APPROVED ALS DRUGS

The pathophysiology of ALS and the mechanisms involved are still poorly understood. Currently, there is no definitive treatment for the progressive neurological disorder ALS and the disease is currently incurable. ALS presents great therapeutic challenges due to the complex underlying pathophysiological mechanisms. Treatment approaches generally focus on symptomatic and supportive therapies. These aim to improve patients' quality of life, alleviate symptoms and prolong life. Medications can be used to reduce muscle spasms, manage psychiatric symptoms such as aggression and depression, reduce salivation and support respiratory function. However, research into potential treatment options for ALS is ongoing. New treatment strategies such as gene therapy, cellular therapy, pharmacological interventions and neuroprotective agents are being studied. To date, only four drugs-riluzole, dextromethorphan hydrobromide with quinidine sulfate, edaravone, sodium phenylbutyrate with taurursodiol, and tofersen - have been approved by the FDA for ALS.

Neurodegeneration in ALS involves a very complex pathological mechanism, including glutamate (Glu) excitotoxicity, free radical formation, cytoplasmic protein deposits, mitochondrial dysfunction with SOD1 enzymes, and disruption of axonal transport processes by accumulation of intracellular neurofilament deposits (Mead et al., 2023). The etiology of ALS includes Glu excitotoxicity, mitochondrial and axonal transport dysfunction, protein aggregation, and increased oxidative stress. To ensure that Glu is removed from synapses and that Glu levels are consequently reduced, riluzole, an FDA-approved drug for the treatment of ALS that slows the rate of disease progression, inhibits Glu transport through the SLC1A3 transporter (Dall'Igna et al., 2013). Research has shown that patients with SALS have lower Glu levels due to a decrease in the Glu receptor GLT1 isoform in the cortex. Riluzole also improves Glu uptake by increasing levels of the amino acid transporter SLC1A1, according to published research (Aykac & Şehirli, 2020).

It is possible to list the following pharmacological properties that may be related to the effects of Riluzole, a Glu antagonist: Inhibition of Glu release, inactivation of voltage-dependent sodium channels, and the ability to interfere with cellular processes following transmitter binding at excitatory amino acid receptors. Glu, the major excitatory neurotransmitter in the CNS, plays an important role in normal nerve cell function. However, excessive release and accumulation of Glu can lead to overactivation of N-methyl-D-aspartate (NMDA) receptors in particular, resulting in the entry of calcium ions into the cell and subsequent nerve cell damage. This process is implicated in the pathophysiology of many neurological diseases, including ALS. Riluzole exerts its neuroprotective effects through several mechanisms. First, it has been shown to inhibit the release of Glu by inhibiting voltage-gated sodium and calcium channels present in glutamatergic neurons at the presynaptic

terminal. By blocking these channels, riluzole prevents excessive Glu release by reducing the entry of sodium and calcium ions. In addition to its effects on glutamatergic neurotransmission, riluzole has other neuroprotective properties. It works by increasing the activity of several antioxidant enzymes, such as SOD, and reducing oxidative stress in neurons. Oxidative stress is another important mechanism implicated in neurodegenerative diseases, including ALS. Clinical studies have shown that treatment with riluzole is associated with a modest prolongation of life in ALS patients. It should be noted, however, that riluzole does not improve patients' quality of life or restore lost motor function. The exact mechanisms of the therapeutic effects of riluzole in ALS are not yet fully understood, and further research should elucidate the precise mechanism of action. In summary, riluzole exerts its neuroprotective effects in ALS by regulating glutamatergic neurotransmission, inhibiting Glu release, antagonizing NMDA receptors, and reducing oxidative stress. Evaluating the effect of riluzole treatment in FUS (1-359) and $SOD1^{G93A}$ mouse model, researchers determined that there was no significant effect on lifespan and motor performance improvement, similar to the other literature results published (Hogg et al., 2018). By investigating the relationships between riluzole treatment and genes effective in ALS, the literature reports that riluzole treatment in transgenic cells did not change the degradation, attenuation, or phosphorylation of the TDP-43 protein (Wright et al., 2021). In the study using a rat transgenic ALS model carrying a mutant human TDP-43 transgene, TDP-43 and ubiquitin deposits were detected in the spinal cords of transgenic rats treated with riluzole. Researchers reported that riluzole treatment did not alleviate behavioral disorders or change neuropathologies (Chen et al., 2020). While it has demonstrated efficacy in prolonging life, its impact on disease progression and functional outcomes in ALS is limited. Further research is needed to unlock the full potential of riluzole and develop more effective treatment strategies for ALS patients.

Riluzole treatment prolongs the life of ALS patients by 2- 3 months, while edaravone delays the progression of the disease by 6 months. Edaravone is a drug that aims to destroy hydroxyl radicals and lipid peroxides by neutralizing free radicals and reactive oxygen species (ROS). Edaravone is thought to act by reducing oxidative damage in neurons, including motor neurons and glial cells, which are vulnerable to damage in ALS (Hardiman et al., 2017). Edaravone reduces the effects of oxidative stress by scavenging free radicals such as hydroxyl radicals, peroxyl radicals, hydrogen peroxide, and ROS. These free radicals accelerate the degenerative process, leading to accelerated nerve cell damage and motor neuron loss. The exact mechanism of action of edaravone in the treatment of ALS is not fully understood. However, it is thought that it may be due to the therapeutic effect of antioxidant activity involved in reducing oxidative stress, which is part of the physiopathology

of ALS. Edaravone's reduction of cellular oxidative stress by scavenging ROS may help protect neurons and prevent motor neuron loss. Clinical trials investigating the effects of edaravone in the treatment of ALS have shown that the drug significantly slows the progression of the disease in ALS patients. These studies showed that edaravone significantly reduced the change in the ALS-Functional Rating Scale over 24 weeks compared to placebo. However, the exact mechanism of action of edaravone in the treatment of ALS is still unclear, and further research is needed. It is believed that other mechanisms, besides the antioxidant activity of edaravone, may be involved. Therefore, further studies and in-depth investigations are needed to fully understand the efficacy of the drug in the treatment of ALS.

Tauroursodeoxycholic acid (TUDCA), or taurursodiol, is a hydrophilic bile acid synthesized by the liver. The neuroprotective effects of the combination of sodium phenylbutyrate and taurursodiol in ALS patients have been reported in many experimental studies. Taurursodiol has been reported to reduce ER stress by inhibiting the mitochondrial apoptosis pathway and oxygen radical production. The results of studies have reported that early initiation of the drug increases survival in ALS patients by 6-7 months (Ketabforoush et al., 2023).

Qalsody (tofersen) is able to reduce SOD1 protein synthesis by promoting the degradation of SOD1 mRNA, and it is emphasized that this drug is more suitable for ALS patients with SOD1 mutations only (Miller et al., 2022). Antisense oligonucleotide (ASO) therapy is a type of gene-targeted therapy that uses short chains of synthetic DNA or RNA molecules that bind to messenger RNA (mRNA) to block the production of specific proteins. In the context of ALS, ASOs can be designed to target mutated genes associated with the disease, such as SOD1. By binding to the mRNA of the mutated gene, ASOs block the translation of the mRNA into toxic proteins, reducing their levels and potentially slowing the progression of ALS. Tofersen (BIIB067), approved under the brand name Tofersen, targets the mutant SOD1 gene and is administered intrathecally directly into the cerebrospinal fluid to block the production of the toxic SOD1 protein. This approach aims to reduce the toxic gain-of-function effects of the mutant protein and provide therapeutic benefits to ALS patients (Saini & Chawla, 2023). In the literature, a decrease in SOD1 expression is reported with the application of tofersen to patients with ALS with SOD1 mutation (Hardiman et al., 2017). In another study, it was determined that intrathecally administered tofersen treatment decreased the synthesis of SOD1 protein when evaluated after autopsy in ALS patients associated with SOD1 ALS mutations (Fang et al., 2022).

Nuedexta (a combination of quinidine sulfate and dextromethorphan hydrobromide) is a sigma-1 (S1) receptor (S1R) agonist and an NMDA receptor antagonist. Quinidine increases the bioavailability

of dextromethorphan hydrobromide by inhibiting the cytochrome P450 enzyme. Nuedexta improves swallowing function in only some patients. Relyvrio (an oral fixed-dose formulation of sodium phenylbutyrate and ursodeoxycholic taurine, AMX0035) plays a role in improving the health of ER and delaying nerve cell death. In the $SOD1^{G93A}$ ALS mouse model, the early onset of the disease and shortening of lifespan caused by deletion of the S1 receptor were reported to be ameliorated by the addition of S1R agonists to the treatment (improvement in motor neuron functions of mice and prolongation of survival) (Jiang et al., 2022; Sun et al., 2024).

CONCLUSION

Only five drugs have received FDA approval to treat ALS. However, current drugs are not enough to increase the survival rate of ALS patients or prevent the progression of the disease. The complexity of ALS pathophysiology makes modelling the disease difficult in both animal and cell experiments and, as a result, poses a serious obstacle to success in the search for a cure. Studies are ongoing to develop drugs that use synthetic organoselenium molecules to stabilize the dimers formed by the mutant SOD1 protein that causes ALS. Drugs such as masitinib and high-dose methylcobalamin are promising, but more data is needed on their long-term effectiveness and safety (Jiang et al., 2022). A better understanding of the mechanisms underlying ALS will enable the development of targeted therapies and, therefore, increase the survival rate of ALS patients. Therefore, it is important to unravel, albeit slowly, the mystery surrounding the genetic and molecular basis of ALS. As with other neurodegenerative diseases, ALS mouse models can provide important information for investigating ALS-related mechanisms and uncovering therapeutic targets. However, the fact that the disease has multiple complicated symptoms and varies from person to person is the biggest obstacle to the positive results obtained from animal models not being obtained at the clinical stage. Therefore, it is recommended to develop additional platforms, such as patient-derived pluripotent stem cells, in preclinical studies.

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Figure Legend

Figure 1. Processes that may be involved in the pathogenesis of ALS

Accumulation of misfolded proteins occurs in the **ER** of sALS patients. Incorrect protein folding within the ER leads to the induction of ER stress and activation of the UPR. The proteostasis network strives to restore protein homeostasis, but its failure can lead to the accumulation of potentially toxic species. Impairment of the proteostasis network is widely observed in the context of ALS pathogenesis. Therefore, ER stress is considered an early pathogenic event in the development of ALS. **Mitochondrial dysfunction** in ALS patients can manifest itself through a number of effects, including disruption of mitochondrial respiratory chain complexes, decreased ATP production and accumulation of reactive oxygen species. Mitochondrial dysfunction may be associated with different mechanisms implicated in the pathogenesis of ALS. These include mitochondrial DNA damage, impaired mitochondrial membrane potential, impaired balance of mitochondrial production and degradation, and impaired mitophagy. Mitochondrial dysfunction, decreased energy production and accumulation of reactive oxygen species can expose motor neurons to oxidative stress and cellular damage, ultimately leading to degeneration. Mitochondrial dysfunction can also trigger neuroinflammation. Mitochondria play an important role in regulating inflammation signaling and their dysfunction can lead to increased inflammatory responses and neuroinflammation. This in turn can accelerate degenerative processes in ALS. **Golgi fragmentation** in ALS has been hypothesized to be initiated by pathogenic mutant proteins that disrupt the normal vesicular trafficking between the ER and Golgi apparatus, as well as the Golgi to plasma membrane transport. This disruption of vesicular trafficking pathways can have several potential consequences in ALS. One consequence is the impairment of autophagy, a crucial cellular process involved in the degradation of misfolded proteins and damaged organelles. Golgi fragmentation may disrupt the proper functioning of autophagy, leading to the accumulation of toxic aggregates and cellular dysfunction. Additionally, Golgi fragmentation can lead to impaired axonal secretory trafficking, compromising the delivery of essential molecules and signaling factors to their intended destinations. This disruption in axonal secretory trafficking can disrupt cellular communication and contribute to the loss of axonal homeostasis observed in ALS. **Nucleocytoplasmic transport** is a mechanism by which genetic material (DNA and RNA) is transported from the nucleus to the cytoplasm and utilized in cellular processes in the cytoplasm. This transport process involves the controlled passage of RNA molecules and RNA-binding proteins from the nucleus across the nuclear membrane into the cytoplasm. The nucleocytoplasmic transport defects observed in ALS patients involve abnormal transport of RNA molecules and

RNA binding proteins from the nucleus to the cytoplasm. These transport defects can cause RNA molecules to incorrectly accumulate intracellularly and fail to reach target cellular sites. Furthermore, abnormalities in the passage of RNA-binding proteins into the cytoplasm can lead to the inability of these proteins to perform their normal functions and dysregulation of cellular processes. **Neuroinflammation** is a process that can contribute to neuronal death through the secretion of inflammatory proteins by activated microglia cells and neurotoxic activation of astrocytes. In ALS patients, activation of microglia and astrocytes is observed as the disease progresses. Activated microglia increase the release of pro-inflammatory cytokines, neurotrophic factors and other inflammatory molecules. When astrocytes are stimulated by inflammatory molecules released by microglia, they can switch to a neurotoxic phenotype. Neurotoxic astrocytes can trigger mechanisms such as glutamate toxicity that can cause damage and death of neurons. **Excitotoxicity** is caused by excessive release of glutamate in nerve cells and this excessive release of glutamate leads to overstimulation of glutamate receptors in neuronal cells, resulting in an abnormal increase in calcium. The increase in calcium ions can lead to impaired mitochondrial function, increased oxidative stress and apoptosis in neuronal cells. **Axonal transport** defects have been shown to be a contributing factor in the pathogenesis of ALS by the presence of neurofilament accumulation and disorganization of cytoskeletal structure in neurons. These abnormalities indicate defects in the proper transport of essential cellular components along axons that may contribute to the progressive degeneration and dysfunction observed in ALS. In ALS patients, astrocytes can become reactive and increase the release of cytokines, neurotrophic factors and other inflammatory molecules. These inflammatory responses can lead to neuroinflammation and damage to motor neurons. Oligodendrocyte dysfunction may contribute to myelin loss, impaired neuronal conduction and degeneration of motor neurons. In ALS patients, excessive activation and inflammation of microglial cells can be observed. This activation can increase neuroinflammation and trigger the release of cytokines, reactive oxygen species and other toxic molecules. Glia dysfunction may involve a number of molecular mechanisms relevant to the pathogenesis of ALS. These include inflammation, cytokine release, neuroinflammation, disruption of glutamate homeostasis, oxidative stress, mitochondrial dysfunction and alterations in neuronal cell death processes. In ALS patients, **dysfunction of Na^+/K^+ ATPase** can be observed. This dysfunction may lead to decreased activity or mislocalization of the Na^+/K^+ ATPase. As a consequence, ion imbalances may occur, such as accumulation of sodium ions inside the cell and lack of potassium ions outside the cell. Impaired Na+/K+ ATPase activity can lead to membrane potential irregularities and impaired nerve conduction. This may contribute to damage and degeneration of motor neurons. Dysfunction of the Na+/K+ ATPase is also associated with other pathological mechanisms.

For example, disruption of Ca2+ homeostasis, oxidative stress, inflammation and disturbances of intracellular calcium balance can trigger or influence the dysfunction of the Na^+/K^+ ATPase. These mechanisms may work in concert with other events associated with the pathogenesis of ALS and the degeneration of motor neurons. Neurofilaments support the structure of the axon and enable the transport of molecules within the axon. In ALS patients, an abnormal accumulation of neurofilaments can be observed. This accumulation can lead to structural disruptions in nerve fibers and damage to neurons. **Neurofilament accumulation** is associated with a disruption of protein homeostasis in nerve cells. In an environment where proteins should normally be structured and functional, misfolded or abnormal proteins begin to accumulate. This accumulation can result from disruptions in the molecular mechanisms involved in the formation and stabilization of neurofilaments. Neurofilament accumulation may also be linked to other pathological mechanisms associated with the pathogenesis of ALS. For example, events such as oxidative stress, inflammation, mitochondrial dysfunction and protein aggregation can trigger or influence neurofilament accumulation. These mechanisms play an important role in the process of damage and degeneration of neurons. **Glia dysfunction** is an important pathological mechanism in ALS. Inflammatory responses of astrocytes, damage to oligodendrocytes and microglial hyperactivation may contribute to ALS progression and motor neuron degeneration. **Vesicle transport** dysfunction may involve a number of molecular mechanisms that may be related to ALS pathogenesis. These mechanisms include protein aggregation, oxidative stress, endoplasmic reticulum stress, inflammation and disruption of intracellular calcium homeostasis.

ER: endoplasmic reticulum, ERAD: Er-associated degradation, UPR: unfolded protein response

Table Legends

Table 1. *ALS-related genes thought to cause ALS or increase the risk of ALS*

Gene	Protein name and function; molecular function and biological process	Locus-Hereditary vs sporadic	Brain expression cluster & RNA expression level in HPA human brain
ALS2 (ALS2CR6)	Alsin Rho guanine nucleotide exchange factor ALS2 Guanine-nucleotide releasing factor Act as a GTPase regulator Controls survival and growth of spinal motoneurons	ALS2-Inheritance recessive, juvenile onset	Non-specific - DNA binding AM, BG, CB*, CC, CP, HF, HT, MO, MB, PN, SC**, TH, WM 11.0 - 64.3
ANG (RAA1, RNASE5)	Angiogenin Protein trafficking, proteostasis, Developmental protein, DNA-binding, Endonuclease, Hydrolase, Nuclease, Protein synthesis inhibitor Angiogenesis, differentiation, stress response	ALS9- Inheritance, dominant, adult onset	Astrocytes - Mixed function AM, BG, CB, CC**, CP*, HF, HT, MO, MB, PN, SC, TH, WM. 1.9-6.4
ANXA11 (ANX11)	Annexin A11 Phospholipid and calcium-binding Cell cycle, Cell division	ALS23- Inheritance dominant, adult -late onset	Choroid plexus - Mixed function AM, BG, CB, CC, CP*, HF, HT, MO, MB, PN**, SC, TH, WM. 39.0 – 102.3
ATXN2 (SCA2, TNRC13)	Ataxin 2 RNA metabolism, DNA repair Involved in EGFR trafficking RNA translation,endocytosis	ALS13- Inheritance, dominant, adult onset	Non-specific- mRNA splicing & Cell cycle AM, BG, CB, CC*, CP, HF, HT, MO, MB, PN, SC**, TH, WM. 48.1 – 83.7
C9orf72 (DENND9, DENNL72, MGC23980)	Chromosome 6 open reading frame 141 C9orf72-SMCR8 complex subunit Protein trafficking, proteostasis; oxidative stress, Guanine-nucleotide releasing factor Autophagy	FTDALS1- Inheritance dominant, adult onset, sporadic	Non-specific – Transcription AM, BG, CB*, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM. 15.2 – 41.5
CAV1 (CAV)	Caveolin 1 neurotrophic and intracellular signaling	ALS-unknown, adult onset	Endothelial cells – Vasculature AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN, SC, TH*, WM. 6.5 – 15.8
CCNF (FBX1, FBXO1)	Cyclin F Protein trafficking, proteostasis; Cell cycle, cell division, Mitosis, Ubl conjugation pathway	FTDALS5- Inheritance dominant, adult onset	Subcortical - Mixed function AM, BG, CB*, CC, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 1.6 - 8.6

CFAP410 (A2, C21orf2, LRRC76, YF5)	Cilia and flagella associated protein 410 role in cilia formation and/or maintenance Cilium biogenesis/degradation, DNA damage	ALS(n) and FTDALS-Inheritance, dominant	Non-specific - Mixed function AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN, SC, TH*, WM. 20.1 – 32.7
CHCHD10 (C22orf16, MIX17A, N27C7-4)	Coiled-coil-helix-coiled-coil-helix domain containing 10 involved in the maintenance of mitochondrial organization and mitochondrial cristae structure	FTDALS2- Inheritance dominant	Choroid plexus - Mixed function AM, BG, CB, CC, CP*, HF, HT, MO, MB, PN, SC**, TH, WM. 132.6 – 327.6
CHMP2B (CHMP2.5, DKFZP564O123, VPS2B)	Charged multivesicular body protein 2B Protein trafficking, proteostasis	ALS17 and Inheritance, dominant	White matter - Myelination AM, BG, CB, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM*. 19.2 – 53.5
DAO (DAAO, DAMOX)	D-amino acid oxidase Regulates the level of the neuromodulator D-serine in the brain Oxidoreductase	ALS- Inheritance dominant, adult onset	Non-specific - Transcription AM, BG, CB*, CC, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 2.2 - 151.3
DCTN1	Dynactin subunit 1 Axo-dendritic transport	ALS and FTDALS-Inheritance dominant, juvenile onset	Neurons & Synapses - Synaptic function, AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN*, SC, TH, WM. 77.2 - 269.0
DNAJC7 (TPR2, TTC2)	DnaJ heat shock protein family (Hsp40) member C7 Acts as co-chaperone Chaperone, protein homeostasis	ALS-Unknown, adult onset	Neurons - Synaptic function AM**, BG, CB, CC**, CP, HF, HT, MO, MB, PN*, SC, TH, WM.40.4 – 55.2
ELP3 (FLJ10422, KAT9)	Elongator acetyltransferase complex subunit 3 Acyltransferase, RNA-binding, Transferase, tRNA-binding Neurogenesis, tRNA processing RNA metabolism	ALS-Unknown, adult onset	Non-specific - Mixed function AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN, SC, TH**, WM. 21.9 – 33.8
ERBB4 (ALS19, HER4)	Erb-b2 receptor tyrosine kinase 4 Activator, Developmental protein, Kinase, Receptor, Transferase, Tyrosine-protein kinase Apoptosis, Lactation, Transcription, Transcription regulation	ALS19-Inheritance dominant and adult onset	Hypothalamus - Neuropeptide signaling AM, BG, CB, CC, CP**, HF, HT, MO, MB*, PN, SC, TH, WM. 12.3 - 63.3
ERLIN1 (C10orf69, Erlin-1, KE04, SPFH1, SPG62)	ER lipid raft associated 1 regulation of cellular cholesterol homeostasis	ALS slow-Inheritance recessive and child onset	Non-specific – Transcription AM, BG*, CB, CC, CP, HF, HT, MO, MB, PN**, SC, TH, WM. 10.3 – 15.1

EWSR1	Ewing sarcoma Breakpoint 1 RNA metabolism	ALS-sporadic	Non-specific – Transcription AM, BG, CB, CC*, CP, HF, HT, MO, MB, PN, SC**, TH, WM. 104.3 – 148.4
FIG4 (ALS11, CMT4J, dJ249I4.1, hSac3, KIAA0274, SAC3)	FIG4 phosphoinositide 5-phosphatase Hydrolase	ALS11-Inheritance dominant, adult onset	Non-specific – Metabolism AM**, BG, CB, CC, CP*, HF, HT, MO, MB, PN, SC, TH, WM. 9.0 – 25.9
FUS (ALS6, FUS1, hnRNP-P2, HNRNPP2, TLS)	FUS RNA binding protein DNA/RNA binding protein, RNA metabolism, transcription; DNA repair DNA-binding, RNA-binding	ALS6- Inheritance minant, recessive or sporadic, adult onset and juvenile	Non-specific - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 33.0 – 92.6
GLT8D1(AD-017, FLJ14611)	Glycosyltransferase 8 domain containing 1 Ganglioside synthesis Glycosyltransferase, Transferase	ALS(n)-Inheritance dominant, adult onset	Choroid plexus - Mixed function AM**, BG, CB, CC, CP*, HF, HT, MO, MB, PN, SC, TH, WM. 28.3 - 53.2
GNE (IBM2, Uae1)	Glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase Allosteric enzyme, Hydrolase, Kinase, Multifunctional enzyme, Transferase	juvenile	Oligodendrocytes - Mixed function AM**, BG, CB, CC, CP, HF, HT, MO, MB, PN, SC, TH, WM*. 16.4 – 26.2
HNRNPA1 (ALS20, hnRNP-A1, HNRPA1)	Heterogeneous nuclear ribonucleoprotein A1 mRNA processing, mRNA splicing, mRNA transport, Transport Ribonucleoprotein, RNA-binding RNA metabolism	ALS20-Inheritance dominant or de novo, adult onset	Non-specific – Ribosome AM, BG, CB, CC, CP, HF**, HT, MO, MB, PN, SC*, TH, WM. 175.0 - 310.8
KANK1 (ANKRD15, KANK, KIAA0172)	KN motif and ankyrin repeat domains 1 Transcription, Transcription regulation, axonopathy	ALS-unknown, adult onset	Immune cells - Immune response AM, BG, CB**, CC, CP*, HF, HT, MO, MB, PN, SC, TH, WM. 27.2 – 56.1
KIF5A (D12S1889, MY050, NKHC, SPG10)	Kinesin family member 5A Motor protein	ALS25- Inheritance dominant, adult onset	Neurons - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 48.0 - 1386.6
LRP12 (FLJ12929, ST7)	LDL receptor-related protein 12 Receptor Endocytosis	ALS28- Inheritance dominant, adult onset	Forebrain - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN**, SC, TH, WM. 55.0 – 93.2
MATR3 (KIAA0723, MGC9105, MPD2, VCPDM)	Matrin 3 RNA-binding RNA metabolism	ALS21- Inheritance dominant; adult onset	Non-specific - Transcription AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 212.2 – 415.1

NEFH (NF-H, NFH)	Neurofilament heavy chain involved in the maintenance of neuronal caliber	ALS-sporadic, adult onset	Non-specific - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 0.5 – 29.5
NEK1 (KIAA1901, NY-REN-55)	NIMA-related kinase 1 kinase, serine/threonine-protein kinase, transferase, tyrosine-protein kinase plays a role in DNA damage repair cell cycle, cell division	ALS24- Inheritance dominant, adult onset	Non-specific - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 33.0 – 92.6
OPTN (FIP-2, FIP2, GLC1E, HIP7, HYPL, NRP, TFIIIA-INTP)	Protein trafficking, proteostasis the maintenance of the Golgi complex, exocytosis Autophagy	ALS12- Inheritance dominant, autosomal recessive, adult onset	Neurons - Mixed function AM, BG, CB, CC, CP**, HF, HT, MO, MB*, PN, SC, TH, WM. 55.0 – 93.2
PFN1 (FIP-2, FIP2, GLC1E, HIP7, HYPL, NRP, TFIIIA-INTP)	Profilin 1 Actin-binding	ALS18- Inheritance dominant, adult onset	Non-specific - Mixed function AM**, BG, CB, CC, CP, HF, HT, MO, MB, PN, SC, TH*, WM. 212.2 - 415.1
PRPH (NEF4, PRPH1)	Peripherin may cooperate with the neuronal intermediate	ALS	Hindbrain - Mixed function AM, BG, CB**, CC, CP, HF, HT, MO, MB, PN, SC, TH, WM*.0.5 – 29.5
SETX (ALS4, AOA2, KIAA0625, SCAR1, Sen1, STEX)	Senataxin Helicase, Hydrolase DNA/RNA metabolism	ALS4- Inheritance dominant, juvenile onset	Non-specific - Transcription AM, BG, CB, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM*. 28.2 - 43.5
SIGMAR1 (OPRS1, SR-BP1)	Protein trafficking, proteostasis, role in BDNF signaling Receptor	ALS16- Inheritance dominant and autosomal recessive	Hypothalamus - Neuropeptide signaling AM, BG**, CB, CC, CP, HF, HT*, MO, MB, PN, SC, TH, WM. 25.0 – 42.1
SOD1 (ALS, ALS1, IPOA)	Superoxide dismutase 1 Destroys radicals that are normally produced within the cells and which are toxic to biological systems Antioxidant, Oxidoreductase	ALS1- Inheritance dominant, adult onset, sporadic, juvenile onset	Non-specific - Mixed function AM**, BG, CB, CC, CP, HF, HT, MO, MB, PN*, SC, TH, WM. 263.5 – 471.4
SPTLC1 (hLCB1, HSAN1, HSN1, LCB1, SPTI)	Serine palmitoyltransferase long chain base subunit 1 Acyltransferase, Transferase Lipid metabolism, Sphingolipid metabolism	ALS27- Inheritance dominant, adult onset, sporadic, juvenile onset	White matter – Myelination AM, BG, CB, CC, CP, HF, HT, MO**, MB, PN, SC, TH, WM*. 263.5 – 471.4
SPG11 (ALS5, FLJ21439, KIAA1840)	Spastic paraplegia 11 SPG11 vesicle trafficking role in neurite plasticity and regulating synaptic vesicle transport	ALS5-juvenile onset	White matter - Signal transduction AM**, BG, CB, CC, CP, HF, HT, MO, MB, PN, SC, TH*, WM. 12.3 - 21.4

SQSTM1 (A170, OSIL, p60, p62, p62B, PDB3)	Sequestosome 1 Protein trafficking, proteostasis; Involved in cell differentiation, apoptosis, and regulation of K^+ channels Autophagy	FTDALS3- Inheritance dominant, adult onset, sporadic	Non-specific – Transcription AM, BG, CB, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM*. 174.7 – 312.6
SS18L1	SS18L1 subunit of BAF chromatin remodeling complex	ALS- Inheritance dominant, adult onset	Cerebellum - Nucleic acid binding AM, BG, CB*, CC, CP, HF, HT, MO, MB, PN, SC**, TH, WM. 27.3 - 66.8
TAF15 (hTAFII68, Npl3, RBP56, TAF2N)	TATA-box binding protein associated factor 15 DNA-binding, RNA-binding RNA metabolism	ALS-sporadic	Choroid plexus - Mitochondria AM, BG, CB, CC, CP*, HF, HT, MO, MB, PN, SC**, TH, WM. 13.3 – 48.3
TARDBP (ALS10, TDP-43)	TAR DNA binding protein RNA-binding protein DNA-binding, Repressor, RNA-binding RNA metabolism	ALS10- Inheritance dominant; inheritance recessive	AM, BG, CB*, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM. 68.5 – 99.8
TBK1 (NAK)	TANK binding kinase 1 Protein trafficking, proteostasis	FTDALS4- Inheritance dominant, adult onset	Subcortical - Mixed function AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN, SC, TH, WM*.6.0 – 9.8
TIA1	Cytotoxic granule-associated RNA-binding protein Stress granule assembly; Axo-dendritic transport; TDP-43 accumulation; RNA metabolism RNA-binding protein involved in the regulation of alternative pre-RNA splicing and mRNA translation	ALS26-Inheritance dominant, adult onset; FTDALS-Inheritance dominant	Non-specific - Nucleic acid binding AM, BG, CB*, CC, CP, HF**, HT, MO, MB, PN, SC, TH, WM. 29.1 – 44.1
TUBA4A (FLJ30169, H2-ALPHA, TUBA1)	Tubulin alpha 4a Hydrolase	ALS22- Inheritance dominant, adult onset	Neurons & Synapses - Synaptic function AM, BG, CB, CC*, CP, HF, HT, MO, MB, PN, SC**, TH, WM. 49.1 - 330.7
UBQLN2 (Chap1, CHAP1/DSK, Dsk2, LIC-2, N4BP4, PLIC-2, PLIC2, RIHFB2157)	Ubiquilin 2 Proteasome Autophagy	ALS15-X-linked Inheritance dominant ALS-X adult onset or juvenile onset	Neurons - Synaptic function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 34.0 – 66.5
UNC13A (KIAA1032, Munc13-1)	Unc-13 homolog A Involved in neurotransmitter release by acting in synaptic vesicle Differentiation, Exocytosis	ALS and FTDALS	Neurons - Mixed function AM, BG, CB, CC*, CP**, HF, HT, MO, MB, PN, SC, TH, WM. 4.6 – 124.2

VAPB (ALS8, VAP-B, VAP-C)	Vesicle-associated membrane protein B Involved in cellular calcium homeostasis regulation Unfolded protein response	ALS8- Inheritance dominant, adult onset	Non-specific - Mixed function AM, BG, CB, CC, CP**, HF, HT, MO, MB, PN, SC, TH*, WM. 23.1 – 60.2
VCP (CDC48, IBMPFD, p97, TERA)	Valosin containing protein Plays a role in the regulation of stress granules clearance process upon arsenite-induced response. Also involved in DNA damage response. Proteasome, vesicle trafficking	ALS14- Inheritance dominant, adult onset, sporadic	Non-specific - Metabolism AM**, BG, CB, CC, CP*, HF, HT, MO, MB, PN, SC, TH, WM. 56.5 - 93.3

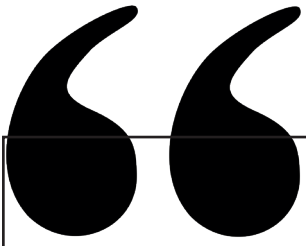
*Region with the highest level of expression; **Region with the lowest level of expression

Hypothalamic-pituitary-adrenal (HPA), amygdala (AM), basal ganglia (BG), cerebellum (CB), cerebral cortex (CC), choroid plexus (CP), hippocampal formation (HF), hypothalamus (HT), medulla oblongata (MO), midbrain (MB), pons (PN), spinal cord (SC), thalamus (TH), white matter (WM).

ALS(n): ALS new

Table 2. *Genes involved in processes involved in the pathomechanism of ALS (Mead et al., 2023)*

Pathogenesis	**Gene name**
Neuroinflammation	*SOD1, PGRN, ERBB4, DAO, TBK1, C9orf 72, OPTN, UBQLN2,*
Proteostasis	*SOD1, VCP, UBQLN2, OPTN, TARDBP, SQSTM1, CHMP2B, FIG4, TBK1, C9orf 72, DNAJC7, TIA1, CCNF, FUS*
Mitochondrial dysfunction	*SOD1, CHCHD10, TARDBP, VCP, TBK1*
Aberrant RNA metabolism	*TARDBP, FUS, SETX, HNRNPA1, ANG, C9orf72, MATR3, TAF15, UBQLN2, ATNX2, TIA1, EWSRI*
Oxidative Stress	*SOD1, ALS2, SQSTM1*
Axonal Dysfunction	*SOD1, DCTN1, SPG11, TUBA4A, PFN1, NFH, VAPB, ALS2, ANXA111, SIGMAR1, C9orf 72, FIG4, SQSTM1, CCNF, KIF5A, KANK1, OPTN, PRN1, TUBA4A*
ER-stress	*SOD1, SIGMAR1, VAPB, VCP*
Dysregulated vesicle & nucleocytoplasmic transport	*CAV1, NEK1, SPG11, FIG4, OPTN, FUS, TARDBP, C9orf72, VABP, ALS2*



Chapter 3

RELATIONSHIP BETWEEN SUBSTANCE P AND DIABETES MELLITUS

Zeynep İpek Salıcı[1]

Çağlar Macit[2]

1 Pharmacist, Istanbul Medipol University, 0009-0002-9765-3464
2 Assistant Professor, Istanbul Medipol University, 0000-0002-5532-2395

1. Introduction

The complications arising from Diabetes Mellitus (DM), which affects 1 in 10 adults and is commonly known as "sugar disease," coupled with the high mortality rate associated with these complications, underscore the need for extensive and costly research into preventive measures and management strategies for this condition. Diabetes mellitus is most generally divided into Type I DM and Type II DM. Since Type I diabetes mellitus is genetic and challenging to prevent, clinical studies have been undertaken to explore preventive strategies for Type II diabetes mellitus.

Substance P, a polypeptide consisting of 11 amino acids, engages with various peripheral nerves and various areas of the central nervous system (CNS). The physiological activity of Substance P (SP), which was first discovered by scientists in 1931, was definitively established in the early 2000s. Research findings indicate that the levels of Substance P is significantly reduced in diabetic neuropathy compared to the control group [1].

SP, which is encountered in almost all systems from the gastrointestinal system to the CNS, from the cardiovascular (CV) system to the endocrine system, has caused clinicians to study it because of its involvement in the complications associated with DM.

Research indicates that Substance P expression is reduced in individuals with diabetes mellitus (DM) compared to those without the condition. This observation has prompted clinicians to explore whether increasing Substance P levels could potentially prevent the complications associated with DM.

This review aims to examine how and through which mechanism substance P is related to DM and the possible results of this relationship.

2. What is substance P?

11 different amino acids are contained in Substance P, present in numerous peripheral nerves and countless areas of the central nervous system. The most remarkable member of the tachykinin neurotransmitter family, which shares the pheglyleum carboxy sequence, to which hemokinin neurokinin A and B also belong, is considered to be it [2],[3]. Tachykinin refers to a family of neuropeptides characterized by a common C-terminal amino acid sequence, while exhibiting variability in their N-terminal sequences. These peptides share functional similarities with Substance P.

Von Euler and his colleagues first discovered Substance P in the horse brain and intestine in 1931 [4]. Although its physiological role was finalized in the early 2000s, the studies conducted about it are older. In the 1950s, it was identified as a neurotransmitter for primary sensory afferent neurons and a neuropeptide with analgesic effects. The clarification of its biochemical

properties occurred in the 1970s. The acceptance of tachykinin as a member of the neurotransmitter family happened in the 1980s

Neurokinin is the term used for those tachykinin family peptides that are exclusive to mammals. NK-1, NK-2, and NK-3 (three types of neurokinin receptors) are also referred to as GPCRs (G-protein-coupled receptors). Substance P functions as a ligand for the NK-1 receptor, which is extensively distributed throughout the body and functions as the primary tachykinin receptor in the human brain. When this receptor is activated, it promotes the development of inositol triphosphate (IP3) and diacylglycerol (DAG). The NK-1 receptor is found in diverse cell types, such as neurons, epithelial cells, adipocytes, and immune cells. [7]-[9]. Therefore, while a deficiency of substance P leads to multiple problems in the body, it can significantly reduce the effectiveness of substance P by affecting this receptor in a disease in the body.

The direct effect on insulin-related signaling is attributed to the interrelation of Substance P with the NK-1 receptor. The responses associated with glucose metabolism are the result of the elimination of this effect [10].

The substance P has a broad range of effects throughout the body, including inducing the vomiting reflex, altering cardiovascular tone, stimulating salivation, and causing vasodilation. Additionally, substance P plays an active role in natriuresis and diuresis carried out by the kidneys, and also in the contraction of venous, intestinal, and bronchial smooth muscles [6].

Vasodilation is the predominant vascular effect of substance P. The majority of substance P's central and peripheral effects for example vasodilation, are mediated through NK1 receptors. The vasodilatory effect of substance P is attributed to its impact on the vascular endothelium, mediated by nitric oxide or other endothelium-derived factors. In addition to vasodilation, substance P also exhibits a vasoconstrictive effect in certain vascular beds [6].

Substance P is involved in regulating the cardiovascular system at central and peripheral levels. Studies have shown that 'nerve fibers' containing Substance P is present in the cardiovascular parts of the central nervous system, consisting the brain and spinal cord. Additionally, fibers containing substance P are located in both the atrial and ventricular muscle tissue of the heart [3],[6],[11].

Diabetes mellitus (DM)-related neurodegeneration causes significant alterations in the overall afferent innervation of the heart, leading to a reduced secretion of CGRP and Substance P (SP) [12].

As diabetes mellitus (DM) advances, the levels of cardiac TRPV1 (transient receptor potential vanilloid 1), CGRP (calcitonin gene-related peptide), and SP (substance P) decline. As a result, the recovery of cardiac function in a

patient undergoing MI is significantly slowed. Activation of TRPV1 prompts the discharge of neuropeptides, such as CGRP and other neurokinins, from sensory nerve terminals. Substance P is classified as a neuropeptide. In the cardiovascular system (CVS), neuropeptides (CGRP and SP) are acting as preservatives [12].

Substance P is densely located in the dorsal horn (substantia gelatinosa). The dorsal horn serves as the initial relay station for main afferent signals, where information is integrated and transmitted to the brain [13]. High levels of Substance P, which is involved in nociception, are found at the terminals of main afferent neurons in the spinal cord, forming the first synapse in the pain transmission pathway within the dorsal horn [3], [6].

In the gastrointestinal tract, Substance P acts as a paracrine agent within the enteric system. Its concentration has been found to vary in proportion with dopamine. It is highly concentrated in the nigrostriatal system (one of the four DOPA pathways in the brain) and the hypothalamus, where it contributes to neuroendocrine regulation. It is involved in various central functions, including stress, anxiety, depression, nausea, and vomiting [6].

Numerous studies have found that Substance P lowers AST levels and other liver function markers, thereby reducing hepatic damage [14].

Finally, the substance P-NK1 system has also been associated with cancer pathophysiology. In clinical studies, it has been found that substance P and NK1 receptors are found in various tumour cells, based on this logic, drug Research has been carried out to explore the idea. that NK1 receptor antagonists have an antitumor effect. NK1 antagonists are highly selective and orally active drugs that can also be passed through BOS. Recent clinical evaluations suggest that these antagonists may be effective in treating depression and other disorders, as well as in preventing chemotherapy-induced vomiting. The first drug approved by this mechanism is Aprepitant. Fosaprepitant, a prodrug that converts to aprepitant following IV administration, serves as a valuable parenteral alternative to oral aprepitant [14].

Substance P is broken down by neutral endopeptidase [14].

3. **Diabetes Mellitus (DM)**

Diabetes is one of the top 10 causes of mortality in the United States. It is a chronic and multifaceted metabolic disorder marked by hyperglycemia. This condition occurs due to either a relative or absolute insulin deficiency or developed insulin resistance in peripheral tissues, known as insulin resistance. It affects many organs and causes multisystemic involvement [15].

Diabetes mellitus (DM), commonly referred to as “sugar disease,” is a significant global health issue affecting millions of people. According to the

most recent study, over 442 million adults worldwide are currently living with DM, and its prevalence has doubled in the last three decades. The Centers for Disease Control and Prevention (CDC) findings that more than 34 million Americans, including 3% of adults, also have DM. These statistics highlight the seriousness of the disease.

Diabetes is diagnosed by evaluating various criteria: a fasting blood sugar (FBS) level of ≥126 mg/dL, an HbA1c level of ≥6.5%, a random glucose level of ≥200 mg/dL in the presence of classic diabetes symptoms, and a 2-hour plasma glucose level of ≥200 mg/dL during an oral glucose tolerance test (OGTT). A patient meeting more than one of these criteria should be assessed for diabetes mellitus (DM) [16].

More than 75% of the total body glucose excretion is excreted from various tissues, including in the brain and peripheral nerves, which do not require insulin. The remaining 25% of glucose metabolism takes place in the liver and muscle, in tissues that require insulin in the direction of glucose uptake into cells [16]. The expression of Substance P in peripheral tissues raises whether or not there is a relationship between these two factors.

Another alarming fact about diabetes is the complications it causes. Eye retinopathy and kidney disease are primarily caused by DM. In addition, a high risk of cardiovascular events, heart failure, and atherosclerotic diseases is faced by patients with DM [16].

The diagnosis of diabetes relies on evaluating several criteria, including a fasting blood sugar (FBS) level of 126 mg/dl, an HbA1c level of 6.5%, a random glucose level of 200 mg/dl, the presence of classic diabetes symptoms, or a 2-hour plasma glucose level of 200 mg/dl during an oral glucose tolerance test (OGTT). A patient meeting more than one of these criteria should be evaluated for diabetes mellitus (DM) [16].

The objectives of diabetes treatment include achieving optimal glycemic control, reducing the onset and progression of diabetes-related complications such as neuropathy, nephropathy, and retinopathy, minimizing cardiovascular risk factors, and enhancing the patient's quality of life.

Currently, the treatment of DM is provided by drugs that work in various mechanisms and by regulating living standards, diet. Unfortunately, DM is an irreversible disease, so the treatment of DM is actually aimed at preventing the complications it will create.

3.1. Type I DM

Type I DM is an inherited disorder. It develops due to the autoimmune destruction of B-cells in the pancreas. These ß-cells are crucial for insulin production. Their destruction results in 'absolute insulin deficiency [18].

In general, it is believed that this disease, which has a fairly low prevalence in society, has turned into a progressive disorder as a result of exposure of genetically sensitive people to an environmental trigger [16].

Type I DM is often diagnosed in childhood or young adulthood. This is because patients in this group experience faster cell destruction. This destruction manifests itself as diabetic ketoacidosis (DKA) [16], [18].

Currently, extensive research is being conducted on the relationship between Type I DM and newly identified autoimmune biomarkers. The goal of these studies is to reduce the exposure of individuals with these markers and to prevent the onset of the disease by identifying these environmental triggers in advance and mitigating their impact [15], [16], [18].

While autoimmunity improves in some individuals, some individuals progress to "absolute β-cell failure, to digitize this sentence, β-cell autoimmunity develops in fewer than 10% of genetically susceptible individuals, only less than 1% of these 10% develop Type I DM [18].

Hyperglycemia is developed only when 60 to 80% of B-cells are destroyed.

This information indicates that Type I DM is a health condition necessitating insulin therapy. Basal bolus insulin therapy via daily multiple insulin injections or continuous subcutaneous (SC) insulin infusion therapy (insulin pump) is considered a treatment options applied by clinicians in Type I DM to ensure optimal glycemic control [18].

In addition to insulin therapy, adjunctive therapies are combined in patients with uncontrolled or irregular glucose October despite insulin therapy [15].

Unfortunately, the applied Type I DM prevention strategies have still not been successful.

3.2. Type II DM

Type II DM is a disruption in insulin secretion resulting from β-cell dysfunction concomitant with insulin resistance. In these patients, the quality and function of the β-cells that a person has due to β-cell dysfunction are severely reduced. Patients with type II DM lose 5 to 7% of the functions of their ß-cells every year. Some clinicians refer to this type of diabetes as non-insulin-dependent diabetes; however, this definition is not full cover for this situation. Because many patients with Type 2 DM experience progressive dysfunction of β-cells in the pancreas, meaning there is a gradual impairment in insulin production. Consequently, people with this condition eventually require insulin therapy.

The onset of this condition is largely influenced by on the living standards of patients. Patients with this disease have almost similar characteristics.

Overweight or obesity... Many studies have shown that many gene mutations affect β-cell development and function, reduce the sensitivity of cells to insulin, or affect the development of obesity [16].

There are multiple defects affecting plasma glucose regulation in this group of patients. Impaired insulin secretion, deficiency of incretin hormones, or the development of resistance are just some of these defects.

Dyslipidemia, characterized by high blood pressure, increased serum triglycerides, and low HDL cholesterol levels, frequently coexists with type 1 diabetes.

Under normal conditions, insulin, primarily secreted by β-cells, is utilized by the helps the body maintain glucose levels within a specific range. It has been observed in studies of overweight individuals without diabetes in which insulin levels boost in direct proportion to the severity of insulin resistance. In this manner, plasma glucose levels were maintained within standard ranges [16].

In managing Type 2 DM, the main treatments concentrate on enhancing insulin secretion, reducing insulin resistance, or achieving glycemic control through both approaches. Understanding the pharmacological properties of antihyperglycemic agents, combined with thorough patient histories, significantly lowers the risk of treatment-induced hypoglycemia. The most effective strategy is to customize treatment for each patient according to the latest clinical guidelines.

For high-risk patients, suspending or even avoiding the onset of the disease can be accomplished through regular exercise, weight management, and dietary modifications. and increased fiber intake. Evidence shows that such measures can lower the risk of developing the disease by up to 60%. [16].

4. **The relationship between DM and substance P**

The relationship between substance P and diabetes mellitus has been carefully studied by clinicians for the last 30 years. The oldest study we have come across on this subject belonged to the year 1997. Given that the physiological role of substance P was identified in the early 2000s, it is reasonable that research on this topic began around that time.

When comparing the concentrations of circulating Substance P between individuals with and without diabetes mellitus (DM), it has been observed that levels are significantly reduced in patients with DM [10], [14], [19]. This observation has led scientists to question what effects might result from externally intervening to preserve or increase these levels. Upon reviewing existing research and clinical studies, it is evident that this relationship is linked to the complications associated with diabetes mellitus (DM).

The contribution of substance P to nociception is known. Based on this information, there are studies on whether substance P deficiency contributes to any nerve damage and what consequences this damage has.

Substance P exerts its effects on diabetes mellitus (DM) mainly through the neurokinin-1 receptor (NK-1R) pathway. NK-1R antagonists, when combined with high-fat diets, promote weight gain in vivo and lead to reductions in circulating insulin and leptin levels [3], [11].

Substance P has a brief half-life (1.6 minutes). Renal peptide elimination decreases with age, impacting substance P stages. In patients with DM, substance P levels decrease 10-30 times more, as DM exacerbates the loss of kidney function [20].

4.1. Substance P and Retinopathy

Retinopathy is an ocular disorder resulting from high blood sugar levels damaging the retina. Chronic inflammation associated with Type II DM induces stress in the body, leading to both micro and macro complications, including retinopathy. The development of diabetic retinopathy varies based hinging on the stage and degree of the diabetes.

Under normal conditions, Substance P accelerates tissue regeneration by replenishing the the stem cell reserve in the bone marrow [21]. It has been found that Substance P can block retinal inflammation and prevent the progression of proliferative vitreoretinopathy (PVR) [22]. These functions of Substance P suggest its potential to impede the progression of diabetes-related complications.

To investigate the effect of Substance P on glucose metabolism, lipid metabolism, systemic inflammation, and retinopathy, Sang-Min Baek and associates conducted a study on rats in 2020, involving 27-week-old OLEFT rats, known for chronic inflammation, obesity, impaired bone marrow secretion, and Type II DM. This age was chosen as diabetic retinopathy typically develops in OLEFT rats after 20 weeks and worsens after 27 weeks. OLEFT rats exhibit spontaneous diabetes, excessive urination (polyuria), excessive thirst (polydipsia), overweight, hypertension, and dyslipidemia [14].

The study delivered Substance P intravenously at a dose of 5 nmol/kg, twice a week for four weeks, to two groups of Type II DM OLEFT rats. One group received Substance P while the other received saline. The effects of Substance P were evaluated at second and forth weeks after injection.

Substance P treatment alleviates chronic inflammation caused by DM by the neurokinin-1 receptor (NK-1R) pathway, by inhibiting glial activation and cell apoptosis, thus promoting diabetic retinopathy healing and preventing retinal damage progression. The beneficial effects of Substance P were observed starting two weeks post-injection and continued for four weeks [14].

4.2. Substance P and Insulin Regulation

Systemic administration of Substance P alleviates impaired glucose regulation in Type II DM rats. Elevated concentrations of free fatty acids (FFA), which have lipotoxic effects on β-cells, increase in Type II DM, thereby inducing insulin resistance. High FFA levels block pancreatic β-cell function, leading to decreased insulin secretion and consequently decreasing glucose uptake in insulin target tissues for instance the liver, skeletal muscle, and heart muscle [23],[24].

A study investigating this effect found that Substance P treatment restored glucose regulation and decreased FFA levels in DM rats, thereby preventing insulin deficiency and insulin resistance. Additionally, Substance P was shown to inhibit pancreatic β-cell destruction, significantly contributing to insulin regulation (pancreatic function restoration) [25].

4.3. Substance P and Wound Healing (Diabetic Foot)

Tissue damage is repaired through wound healing, a coordinated process that restores and regenerates damaged tissue. This process comprises a well-organized series of biochemical steps. Wound healing occurs in four stages: hemostasis, inflammation, cell proliferation, and tissue regeneration [1]. Each stage is tightly regulated by different cell types and signaling molecules. Relevant cells release a spectrum the release of multiple growth factors and cytokines at the wound site, orchestrating the wound healing process [26],[27].

Diseases such as diabetes, venous insufficiency, or autoimmune disorders can disrupt the wound healing process, potentially resulting in chronic wounds that do not heal. In advanced stages, diabetes can lead to chronic wounds that fail to progress through the standard stages of wound healing in a timely and regular manner [21], [26], [28]. These wounds, commonly occurring in the oral cavity and on the base of the feet, administer a significant economic burden on the healthcare system because of their difficult healing process. Consequently, clinicians are engaged in efforts to prevent the formation of such wounds.

Substance P is one of the primary neuropeptides released from nerve fibers as a consequence of injury and is extensively studied for treating various wound types [1].

Despite the main mechanism is not fully understood, numerous studies suggest that Substance P plays a vital role in tissue regeneration [1], [29].

In damaged tissue, sensory nerve fibers and inflammatory cells secrete Substance P [31], [32]. To carry out its functions in skin repair and regeneration, Substance P interacts directly with various cells and molecular pathways, and indirectly with NK-1R on nerves, epithelial cells, and various inflammatory cells (mast cells, macrophages, T lymphocytes). Recent findings indicate that

Substance P accelerates the wound healing process at all stages, especially by modulating the inflammatory phase [1], [29].

4.4. Substance P and Neuropathy

Neuropathy refers to conditions resulting from damage to nerves or nerve fibers. A study assessing the function of Substance P in diabetes mellitus (DM) and DM-related neuropathy involved 75 healthy volunteers and 50 Type I DM patients (1-35 years duration). The DM patients included those with retinopathy and/or neuropathy. At the outset of the study, serum and plasma samples were acquired from participants to evaluate Substance P levels in the serum.

Comparing serum Substance P levels revealed that Type I DM participants had significantly lower serum Substance P levels than the control (healthy) group ($p<0.0001$). Additionally, among DM participants, those with neuropathy had significantly lower serum Substance P levels than those without neuropathy ($p<0.04$). This finding suggests that Substance P is reduced in peripheral nerves in diabetes mellitus (DM).

In neuropathic DM patients, decreased Substance P levels were strongly associated with damage to Aδ- and C-fibers in the lower extremities, as measured by computer-assisted thresholds for vibration, heat, and pain perception [20].

The development of sensory polyneuropathy in DM patients is often attributed to functional and structural changes in nociceptive neurons [5]. Sensory fiber degeneration, a frequent complication of diabetes, is seen in both diabetic animals and humans.

A study identified a notable reduction in SP and CGRP release in diabetic mouse hearts, correlating with the development of diabetic autonomic neuropathy [33].

5. Discussion

New studies are necessary to assess the direct effects of Substance P on insulin resistance and adipocyte-associated lipid metabolism. Research findings highlight that insulin-induced glucose uptake in adipocytes is inhibited by Substance P. Considering the contribution of adipose tissue in insulin resistance, this peptide could appear as a potential target for novel therapeutic approaches designed to tackle insulin resistance.

Substance P has a brief half-life (1.6 minutes) [1], so future research should focus on synthesizing modified analogs with enhanced stability to increase Substance P's stability. Substance P holds potential as a marker for the onset of DM neuropathy, although additional research in this field is essential.

Recent studies has demonstrated that Substance P alleviates impaired glucose regulation. By modulating the inflammation phase, Substance P accelerates all phases of the wound healing process.

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Chapter 4

ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE*

Kerim Kaan GÖKÜSTÜN[1]

1 * This study was derived from the thesis titled "Evaluation of the effects of n-acetylcysteine on atherosclerosis and oxidative stress parameters in mice fed with Western diet". (Advisor: Prof. Dr. Nurcan YABANCI AYHAN; Co-advisor: Assistant Professor Onder OTLU.

Research Assistant Dr. Kerim Kaan GÖKÜSTÜN, Malatya Turgut Özal University, Faculty of Health Science, Department of Nutrition and Dietetics, kerimkaangokustun@ozal.edu.tr, 0000-0001-6725-4388.

Introduction

Cardiovascular disease (CVD) is a serious health problem characterized by deterioration of the cardiovascular system. Hypertension, ischemic heart disease, congenital heart diseases, cerebrovascular diseases, coronary heart diseases, arrhythmias, rheumatic heart diseases, peripheral vascular disease, heart failure and atherosclerosis are called CVD (Dülek, Tuzcular Vural, & Gönenç, 2018). The World Health Organization (WHO) states that CVD is the most important cause of morbidity and mortality. Cardiovascular diseases are responsible for approximately 38% of deaths due to non-communicable diseases in individuals under 70 years of age in 2019 (WHO, 2021). In our country, CVD mortality rate was found to be approximately 50% in 2018 (Kayikcioğlu & Oto, 2020).

Atherosclerosis is the most important pathophysiological pathway underlying myocardial infarction, stroke and many cardiovascular diseases (Poznyak et al., 2021). This study aims to clarify the mechanism of atherosclerosis and its role in cardiovascular disorders.

Risk Factors for Cardiovascular Diseases

It is considered that over 300 risk factors have a potential to cause cardiovascular diseases (Oğuz, Erguvan, Ünal, Bayrak, & Çamcı, 2019). There are two types of risk factors: modifiable risk factors and non-modifiable risk factors (Türker, 2013). Modifiable risk factors include smoking and excessive alcohol use, dyslipidemia, obesity, physical inactivity, hyperhomocysteinemia, diabetes, poor sleep quality, western-style diet, metabolic syndrome, inflammation, elevated serum fibrinogen, and hypertension. Non-modifiable risk factors include male gender, being older than 45 years old for men and older than 55 years old for women, as well as a family history of cardiovascular disease. (Oğuz et al., 2019; Türker, 2013). One of the most significant risk factors is nutrition (Doğan & Kartal, 2019). It has been discovered that a problematic diet, an increase in daily energy intake, an excessive intake of foods derived from animals, a poor intake of fruits and vegetables, and a diet excessive in sugar and saturated fat are linked to an elevated risk of CVD. (Doğan & Kartal, 2019; Mertens, Markey, Geleijnse, Givens, & Lovegrove, 2017).

Pathophysiology of Atherosclerosis

The name "atherosclerosis" is a combination of the ancient Greek words "athere" meaning porridge and "sclerosis" meaning stiffening. Atherosclerosis is when the arterial walls are covered with a soft substance resembling porridge and the substance stiffens the artery, narrowing the vessel and preventing blood flow. Atherosclerosis is defined as the thickening and stiffening of any blood vessel (Türker, 2013). Atherosclerosis is the major contributor to coronary artery, carotid artery and peripheral artery disease. Although atherosclerosis

is generally not life-threatening on its own, the addition of many factors to atherosclerotic plaque in damaged arteries can lead to fatal health problems such as acute coronary syndrome and stroke (Falk, 2006).

A normal, healthy artery consists of three layers.

1- Intima layer: It is composed of endothelial cells, which lining blood vessel lumen.

2- Media layer: This layer contains smooth muscle cells and elastin fibers that control arterial tone.

3- Adventitia layer: This layer is surrounded by connective tissue containing microvessels and is associated with perivascular adipose tissue (Douglas & Channon, 2014).

Atherosclerosis is primarily caused by free radical-mediated oxidation of low-density lipoproteins (LDL-C), activation of various cell types, chemoattractants, and endothelial function degradation (Tousoulis et al., 2011). Atherosclerosis is also known as vascular endothelial damage leading to endothelial dysfunction. Endothelial dysfunction is widely believed to be one of the most prominent factors responsible for the initiation of atherogenesis, contributing to the process of atherosclerotic plaque formation (Sitia et al., 2010). Increased blood pressure and serum LDL-C levels, impaired carbohydrate metabolism, and disruption of carbohydrate metabolism lead to malfunction of endothelial cells, i.e. endothelial dysfunction (Türker, 2013). The production and synthesis of reactive oxygen species are promoted by disruption of endothelial cell function, which also has an adverse effect on nitric oxide biosynthesis and bioavailability, which is the mediator of vasodilation. These conditions facilitate the formation of atherosclerotic lesions (Tousoulis et al., 2011).

Hypercholesterolemia is one of the most important triggering factors of atherosclerosis. Damaged vascular endothelium and elevated plasma cholesterol levels lead to altered endothelial permeability. This situation allows the migration of lipids, especially LDL-C, into the arterial walls. Circulating monocytes adhere to endothelial cells and cause the release of adhesion molecules such as selectin and vascular cell adhesion molecule-1 (VCAM-1) (Sakakura et al., 2013). These molecules migrate into the subendothelial space. After entering the subendothelial space, monocytes become macrophages and transform into foam macrophage cells. LDL-C particles in the subendothelial space are oxidized and transform into potent chemoattractants (Bergheanu, Bodde, & Jukema, 2017). Macrophages take up oxidized LDL, other atherogenic lipoproteins and phospholipids, increase the expression of scavenger receptors (A, B1, CD36, CD68) and contribute to foam cell formation. Atherosclerosis is encouraged by smooth muscle cells that also migrate to the endothelium layer.

(Bergheanu et al., 2017; Fan & Watanabe, 2022).

Atherosclerosis and Inflammation

It has long been well known that systemic and local inflammation plays an important role in the pathogenesis of atherosclerosis, the development and progression of cardiovascular diseases. Inflammatory factors such as psychological stress, autoimmune diseases, microbial and viral infections and aging trigger inflammation, activate endothelial damage and dysfunction and contribute to the process of atherosclerosis (Henein et al, 2022). Several inflammatory mechanisms can contribute to atheroma plaque formation (Geovanini & Libby, 2018). First, various factors causing inflammation decrease the bioavailability of nitric oxide (vasodilator) released from the endothelium, increase the release of endothelin (vasoconstructor) and damage endothelial junctions. These changes facilitate LDL-C transition to the subendothelial space. When LDL-C enters the endothelial space, it undergoes oxidation and thus the inflammatory process begins. Oxidized LDL-C is phagocytosed by macrophages. Macrophages are then transformed into monocytes. Monocytes secrete inflammatory cytokines, including TNF-α, IL-1α, IL-1β, IL-12, IL-15, and IL-18 (Henein et al, 2022). Leukocytes and proinflammatory cytokines play an important role in the early phase of atherogenesis (Geovanini & Libby, 2018). When endothelial cells are activated, selectin, intracellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1) and other inflammatory factors interact with lymphocytes and monocytes, bind to the endothelium, enter the arterial wall and inflammation initiates (Chistiakov, Melnichenko, Grechko, Myasoedova, & Orekhov, 2018).

Atherosclerosis and Oxidative Stress

For many years, it has been understood that oxidative stress is a major factor in the development of atherosclerosis. Endothelial cell dysfunction is triggered by the production of free radicals. These radicals initiate the oxidation of LDL-C. (Ketelhuth & Hansson, 2011). Compared to unoxidized LDL-C, macrophages are more prone to absorb oxidized LDL-C. In atherosclerotic vessels, macrophages and vascular smooth muscle cells are two of the main causes of oxidative stress. Vascular smooth muscle cells have the ability to produce superoxide radicals when their blood cholesterol levels are high. This increases LDL-C oxidation. The synthesis, activity, and bioavailability of nitric oxide are also negatively impacted by elevated ROS generation. Reduced nitric oxide synthesis and bioavailability can lead to platelet aggregation, increased vasoconstriction, decreased vasodilation, and diminished neutrophil adherence to the endotelium. (Kattoor, Pothineni, Palagiri, & Mehta, 2017; Vogiatzi, Tousoulis, & Stefanadis, 2009).

Atherosclerosis and Obesity

Abdominal obesity may accelerate the progression of atherosclerosis. Atherosclerotic plaque formation due to vascular damage in coronary arteries is associated with total and abdominal obesity. Adipokines are thought to explain the relationship between obesity and atherosclerosis (Koliaki, Liatis, & Kokkinos, 2019). The equilibrium between energy intake and expenditure triggers the release of anti-inflammatory adipokines from adipocytes, including IL-10, adiponectin, TGF-β, and NO. These adipokines have antiatherogenic properties (Han, Quon, Kim, & Koh, 2007). Adiponectin is thought to be the most fundamental anti-atherogenic adipokine (Katsiki, Mantzoros, & Mikhailidis, 2017). These adipokines enhance endothelial function by producing more NO and prevent macrophages from converting into foam cells (Ouchi et al., 2004). However, dysfunctional hypertrophic adiposity characterized by excessive energy intake causes the release of pro-inflammatory adipokines with atherogenic effects such as TNF-α, leptin, IL-6, resistin, IL-18, retinol binding protein-4 and lipocalin-2. Hyperleptinemia caused by excess adipose tissue and hypothalamic leptin resistance can cause vascular inflammation, left ventricular hypertrophy, systemic insulin resistance oxidative stress and atherothrombosis (Koliaki et al., 2019).

Increased body fat is linked to a malfunction in the metabolism of lipids and lipoproteins, which raises plasma cholesterol and LDL-C while lowering HDL-C. Both the risk of ischemic cardiomyopathy and the advancement of atherosclerosis are raised by these situations (Piché, Poirier, Lemieux, & Després, 2018). Moreover, it raises blood pressure, worsens glucose intolerance, puts more strain on the left ventricular wall, and raises the risk of heart failure, atrial fibrillation, coronary artery disease, and sudden cardiac death (Piché, Tchernof, & Després, 2020).

Atherosclerosis and Type 2 Diabetes

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by chronic hyperglycemia caused by problems in insulin secretion, insulin efficacy or both. Insulin resistance in target tissues and insufficient insulin secretion lead to metabolic complications in individuals with diabetes (Poznyak et al., 2020).

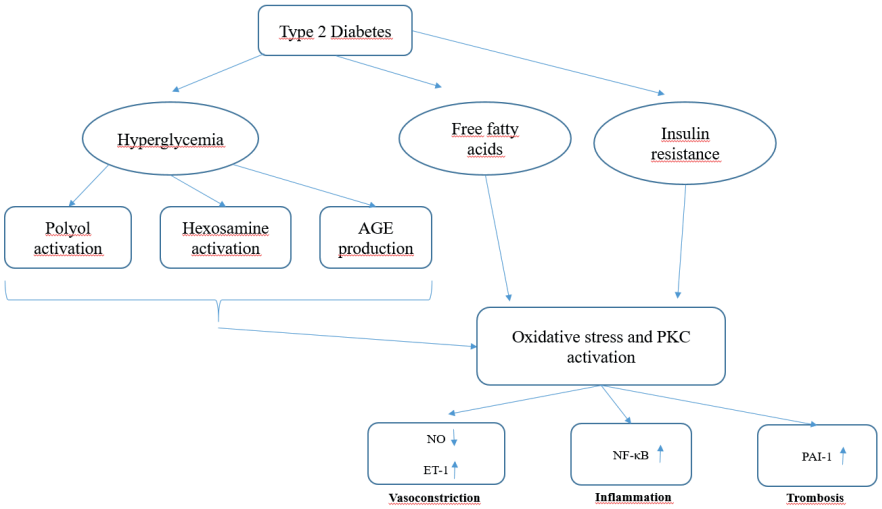
One of the most important causes of atherosclerotic cardiovascular diseases is hyperglycemia and insulin resistance (Bornfeldt & Tabas, 2011). Approximately 75% of diabetic individuals die due to atherosclerosis (Hurst & Lee, 2003). Hyperglycemia, excessive increase in free fatty acids and insulin resistance lead to an increase in Advanced Glycation End Products (AGE), activation of Receptors for Advanced Glycation End Products (RAGE) and impairment of Protein Kinase C signaling (Henning, 2018). These results in decreased NO synthesis and impaired vascular endothelial cell function.

Disruption of Protein Kinase C signaling increases vasoconstriction and leukocyte adhesion. Increased activation of receptors for advanced glycation end products (RAGE) increases endothelial superoxide radical production (Petersen, Dufour, Befroy, Garcia, & Shulman, 2004; Schmidt & Stern, 2000). These decreases NO synthase activity. In addition, increased serum glucose and free fatty acids contribute to mitochondrial dysfunction and promote ROS production and insulin resistance; increase NF-κβ activation, endothelin-1 (ET-1) production, which is associated with vasoconstriction; and stimulate the release of other factors associated with vascular inflammation, vasoconstriction and thrombosis (Henning, 2018).

Approximately 97% of diabetic individuals suffer from dyslipidemia. Dyslipidemia is a risk factor for atherosclerosis. In individuals with diabetes, LDL-C particles are smaller and denser than in healthy individuals. Small and dense LDL-C particles pass through arterial walls more easily, oxidize and adhere more strongly to arterial walls (Henning, 2018).

It is known that oxidative stress is increased in individuals with type 2 diabetes. Among the factors that lead to elevated oxidative stress in diabetics are elevated glucose oxidation, elevated glycation, AGE-RAGE axis activation, polyol pathway activation, and antioxidant system impairment. Elevated oxidative stress levels cause endothelial cell dysfunction, increase coagulation tendency, destabilize atherosclerotic plaque, and cause vascular smooth muscle cell migration, proliferation, and transformation. Pro-inflammatory reactions and the release of pro-inflammatory cytokines are also triggered by them. This expedites the process of atherosclerosis progression (Katakami, 2017).

Figure 1. *The relationship between type 2 diabetes and atherosclerosis*



AGE: Advanced glycation end products; NO: Nitrik oksit; ET-1: Endotelin-1; NF-Kβ: Nükleer faktör kappa beta; PAI-1: Plazminojen aktivator inhibitor-1; PKC: Protein Kinase C

Atherosclerosis and Hyperhomocysteinemia

Hyperhomocysteinemia is an emerging risk factor for atherosclerosis, including type 2 diabetes, hypercholesterolemia and hypertension. Homocysteine is produced as a transitional step from methionine to cysteine. The amino acid can be converted to cysteine via the transsulfuration pathway or methionine can be re-synthesized from homocysteine via the remethylation pathway (Lawrence de Koning, Werstuck, Zhou, & Austin, 2003).

Hyperhomocysteinemic individuals may often develop fibrous plaques, or arterial intimal thickening, rich in collagen and smooth muscle. These fibrous lesions may be much more abundant than lipid-induced atherosclerotic lesions in the main arteries of homocystinuric patients and may lead to tissue infarction with abnormally accelerated thrombosis and premature death (Lawrence de Koning et al., 2003).

Homocysteine may contribute to atherosclerotic lesion formation by enhancing the inflammatory response and oxidative stress. This amino acid can promote the production of MCP-1, IL-8 cytokines and activate the transcription of NF-κB. It can also damage endothelial cells and disrupt normal cell function and vasodilation (Lawrence de Koning et al., 2003).

It has been suggested that homocysteine may undergo auto-oxidation, thus contributing to the production of reactive oxygen species and leading

to cell dysfunction. However, these hypothesis was found to be inaccurate since cysteine, whose plasma level is 20-30 times higher than homocysteine and can be more easily auto-oxidized, does not cause endothelial cell damage. (Lawrence de Koning et al., 2003).

Homocysteine may trigger oxidative stress through different mechanisms. Since homocysteine inhibits the expression of antioxidant enzymes and increases the formation of superoxide anion, it may exacerbate oxidative stress. Superoxide anion can react with NO and decrease the bioavailability of NO. Decreased bioavailability of nitric oxide may increase the expression of MCP-1 and accelerate atherosclerotic lesion formation (Lawrence de Koning et al., 2003).

Atherosclerosis and the Western Diet

Numerous non-communicable diseases, including heart disease, are influenced by unhealthy eating patterns. Western-style diet, which is a diet high in fat (especially saturated fat) and refined carbohydrates, increased consumption of fast-food style foods and beverages with high sugar content, plays an essential role in the development of cardiovascular diseases (Jacka et al., 2015).

High dietary fat intake can be associated with atherosclerosis. Saturated fatty acids have played an important role in the diets of western countries for centuries. These fatty acids have a terrible reputation due to their known negative effects on cardiovascular diseases (Feinman, 2010). Saturated fats may raise the risk of myocardial infarction and cardiovascular disease by lowering high-density lipoprotein (HDL-C) in the blood and raising levels of LDL-C and triglycerides, which may predispose to atherogenic dyslipidemia (Feinman, 2010). Studies have shown that consumption of polyunsaturated fatty acids instead of saturated fatty acids can reduce plasma cholesterol and LDL-C levels and the rate of LDL-C production in hyperlipidemic individuals. It has also been reported that reducing saturated fatty acid consumption can also decrease the risk of cardiovascular events by approximately 17% (Hodson, Skeaff, & Chisholm, 2001; Hooper et al., 2020; Siri-Tarino, Sun, Hu, & Krauss, 2010).

Saturated fatty acids are thought to have adverse effects on endothelial cells by activating inflammatory signaling pathways. Excess dietary intake of saturated fatty acids stimulates the release of pro-inflammatory mediators such as TNFα, IL-1β, IL-6, IL-12, interferon γ (IFN-γ) and MCP-1 via toll-like receptor-4 (TLR-4) (Tamer, Ulug, Akyol, & Nergiz-Unal, 2020). Especially myristate and palmitate, known as long-chain fatty acids, can induce apoptosis by activating NF-κB in coronary artery endothelial cells. Stearic acid, on the other hand, can cause up-regulation of ICAM-1 in aortic endothelial cells via the NF-Kβ signaling pathway. In addition, long-chain fatty acids can induce

pro-inflammatory endothelial cell phenotypes by interacting with endothelial cell lipids (Muñoz & Costa, 2013).

Excessive dietary intake of refined carbohydrates is an elevated risk factor for cardiovascular diseases (Temple, 2018). Increased incidence of obesity, especially with excessive intake of sugar and refined carbohydrates, paves the way for the formation of atherogenic dyslipidemia, which is characterized by an increase in triglycerides, small and dense LDL-C particles and a decrease in HDL-C concentrations (Siri-Tarino et al., 2010). Studies have reported that refined carbohydrates are an independent risk factor for cardiovascular disease and may elevate the risk of these diseases by up to 25% (Barclay et al., 2008; Tanasescu, Cho, Manson, & Hu, 2004).

It is well known that an essential function of the pathophysiology of cardiovascular disease is mediated by foods and beverages with added sugar, such as table sugar, brown sugar, corn syrup, and high-sugar beverages. These foods increase the synthesis of reactive oxygen species (ROS) in mitochondria through the uric acid, insulin, polyol, glycation activation, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathways (Prasad & Dhar, 2014).

Atherosclerosis and Sleep

Poor sleep quality, short sleep duration and sleep fragmentation are associated with cardiovascular risk factors, increased incidence of cardiovascular disease and mortality from cardiovascular disease (Full et al., 2023). It is considered that sleep quality and duration may directly or indirectly affect the process of atherosclerosis development. Sleep restriction elevates serum levels of C-reactive protein, interleukin-8, myeloperoxidase, fibrinogen and apolipoproteins (Bhagavan & Sahota, 2021). In a study, very short sleep duration was reported to increase the risk of atherosclerosis by approximately 27% (Lim, 2019). Sleep fragmentation may lead to sympathetic nervous system activation, endothelial dysfunction, increased oxidative stress and inflammation (Bhagavan & Sahota, 2021). Short sleep duration and poor sleep quality promotes the development of obesity by increasing food intake. It also raises the risk of diabetes by 30%, hypertension, dyslipidemia, chronic kidney disease (Domínguez et al., 2019; Kadoya & Koyama, 2019).

Atherosclerosis and Smoking

The development of atherosclerosis is independently correlated with smoking. Cigarette smoke has thousands of hazardous chemicals including tar, formaldehyde, carbon monoxide, acetaldehyde and nicotine. Cigarette smoke exposure was found to be associated with dyslipidemia, hyperglycemia, insulin resistance, vascular inflammation and angiogenesisIt also alters the homeostasis of endothelial cells and facilitates the development of atherosclerosis (Fu et al.,

2021). It also accelerates the process of coronary atherosclerosis by increasing LDL oxidation and damaging coronary endothelial vasodilation (Salehi, Janjani, Tadbiri, Rozbahani, & Jalilian, 2021).

Nicotine is an important molecule that accelerates the formation of atherosclerosis. It causes vascular endothelial dysfunction, initiates adhesion cascade and stimulates vascular inflammatory response. Nicotine damages vascular endothelium by increasing oxidative stress and lipid peroxidation. Nicotine also increases the release of IL-1β, IL-6, and TNF-α, increases the release of adhesion molecules and accelerates the adhesion of monocytes (Fu et al., 2021).

Atherosclerosis and Alcohol

Although alcohol consumption is known to be a risk factor for cardiovascular diseases, it has been reported to have both beneficial and detrimental effects on the progression of atherosclerosis. Low or moderate alcohol consumption reduces the risk of cardiovascular diseases such as coronary heart disease and ischemic stroke. However, excessive alcohol consumption is associated with an increased risk of cardiovascular disease. When the protective mechanisms of alcohol consumption are examined, it is demonstrated that it accelerates glucose metabolism, increases serum HDL-C levels and exhibits anti-inflammatory effects. However, it is considered that alcohol consumption may predispose to cardiovascular diseases by increasing serum triglycerides, glucose and blood pressure (Kim et al., 2014).

The type of alcoholic beverages is also very important for cardiovascular diseases. For example, the French have been reported to have low mortality rates from cardiovascular diseases despite their consumption of high-fat foods and excessive smoking. It is thought that this is because the French consume large amounts of wine. These situation is known as the French paradox. It is believed that resveratrol in wine reduces the risk of cardiovascular disease by inhibiting platelet aggregation and slowing the oxidation of LDL-C (Tolstrup & Grønbaek, 2007).

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Chapter 5

THE RELATIONSHIP BETWEEN TOXOPLASMA GONDII PARASITE AND PSYCHIATRIC DISEASES: INVESTIGATION OF POSSIBLE LINKS BETWEEN PARASITIC INFECTION AND SCHIZOPHRENIA

Bashar IBRAHIM[1]

Ahmet Arif KURT[2]

1 Dr.Öğr.Üyesi
2 Dr.Öğr.Üyesi

INTRODUCTION

Current research suggests that exposure to infections in childhood is associated with the later development of schizophrenia (1). Schizophrenia is a public health issue that typically starts in adolescence. Its course and outcome vary from patient to patient and it shows signs and symptoms affecting all areas of mental health. Ongoing research is being conducted to explore the potential connection between *Toxoplasma gondii* (*T. gondii*) and schizophrenia, as well as other psychiatric illnesses. In addition to *T. gondii*, various infectious agents such as herpes viruses, varicella Zoster virus (VZV), and Epstein-Barr virus (EBV) have been proposed to be involved in schizophrenia. These microorganisms, which share a common characteristic, may have a latent period and persist in the host for an extended duration (2). In recent years, *T. gondii* has been the most extensively studied among these agents and has shown high levels of serum antibody presence. Studies, particularly on first-episode schizophrenia patients, have demonstrated the presence of excess *T. gondii* IgG antibodies in the serum (3). Toxoplasma, an intracellular protozoa, is a widespread parasite that significantly affects human health. It involves a significant portion of the world's population in developed and developing countries. The method of transmission varies among different populations. It occurs due to the dietary habits of 80% of the population and contact with cats, the final host. There is a latent period after the initial infection and most cases are observed asymptomatically. However, there is a risk of congenital transmission (4). The specific localization of latent *T. gondii* infections to central nervous system (CNS) cells is considered the most important feature of this parasite. This allows it to cause cellular and/or secretory issues without displaying the typical signs of infection during the latent phase (5). It has been observed that 60% of latently infected and immunocompromised individuals experience disorientation, anxiety, depression and schizophrenic psychosis as a result of toxoplasmosis. Similarly, psychiatric complications and meningoencephalitis can also be seen in patients infected with toxoplasmosis. Schizophrenia is a serious neuropsychiatric disorder of unknown etiology, and although genetic factors are thought to play an important role, epidemiological evidence suggests that in some cases infectious diseases may also be associated with schizophrenia (2,6). Furthermore, as an analogy to the familial transmission of genetic material in schizophrenia, animal models have demonstrated that genes can influence susceptibility to the parasite in *T. gondii* infections. Studies have shown that patients with major depressive disorder (MDD) or those showing symptoms of anxiety and depression are known to have elevated levels of CD4+ T in their serum (7). It has been definitively demonstrated that mice infected with *T. gondii* over an extended period are capable of transmitting the infection to five consecutive generations (8). The clinical symptoms of

toxoplasmosis are still not fully understood, but there is evidence suggesting that the parasite may be involved in neurodevelopmental processes and the onset of schizophrenia. This is linked to the parasite's preference for glial cells in the brain, which is believed to contribute to the development of the disease (9)(10). Binbay and colleagues conducted a systematic review of the literature on immigrants of Turkish origin residing in the Netherlands, Germany, Denmark, and Switzerland. The systematic evaluation of articles published between 1990 and 2010 revealed that the frequency of all psychotic disorders, affective and non-affective, and the frequency of schizophrenia in immigrants of Turkish origin exhibited considerable variation, with rates ranging from 38.5 to 44.9 per hundred thousand and 12.4 to 63.8 per hundred thousand, respectively. A review of the literature reveals that schizophrenia and other psychotic disorders affect 1.1 to 6.2 out of every 1,000 people (10). Recent studies have indicated that *T. gondii*, a neurotropic parasite, may contribute to the pathogenesis of schizophrenia. This is evidenced by the observation of behavioral changes, suicidal ideation, and neuropathological degeneration in brain tissue (7). Additionally, it has been suggested that *T. gondii*, present in approximately one-third of the global population, may be associated with an increase in suicide rates across various European countries (11)(12). Literature review reveals that studies examining *T. gondii* antibodies in schizophrenia patients in Turkey have reported seropositivity rates for IgG antibodies between 40% and 66% (13). This study aims to investigate the potential links between the Toxoplasma gondii parasite and psychiatric disorders, including schizophrenia.

Life Cycle

Members of the family Felidae are the definitive hosts of *T. gondii*. Cats, mice, and rats become infected with *T. gondii* by somehow ingesting the parasite. Once inside the digestive system, the parasite enters the cells lining the intestines. Through a form of asexual reproduction called schizogony, the parasite multiplies, producing an average of 10–16, or 2–40 merozoites. Sexual reproduction, or sporogony, leads to the production of oocysts. During this process, microgametocytes and macrogametocytes are created within 3–15 days and then mature into macrogametes (14,15). The zygote is formed when the male cell, the microgamete, fertilizes the female cell, the macrogamete. The zygote undergoes maturation into immature oocysts within four days, after which it is excreted initially in the intestinal lumen and subsequently in the feces (15). Following the ingestion of mature oocysts by a cat, the subsequent excretion of immature oocysts in the feces occurs approximately three weeks later. The ingestion of mice with trophozoites by the cat, occurring ten days after the initial ingestion of mature oocysts, results in the excretion of immature oocysts after a further ten days. The ingestion of mice with cysts (bradyzoites) by the cat, occurring between three and five

days after the initial ingestion of mature oocysts, also results in the excretion of immature oocysts after a further one to two weeks. In the initial 1–3-week period, a cat with an acute infection can excrete 10^7–10^9 oocysts per day (16). It has been demonstrated that sporozoites present in mature oocysts, trophozoites in infected animals, and bradyzoites in humans are infectious to cats and other susceptible hosts, including humans. Oocysts have been demonstrated to remain infective in soil for 18 months (17).

Epidemiology of *Toxoplasma gondii*

T. gondii is a parasite that affects approximately one-third of the global population and is particularly prevalent in developing countries (4). Following the initial acute infection, the parasite enters the latent period. Depending on eating habits and contact with cats, 80% of the population may be infected. Latent infection with *T. gondii* is the most common infection in humans but is usually asymptomatic except for congenital transmission. It has been reported that latent infections can cause behavioral changes in rodents (18). *T. gondii* is an obligate intracellular protozoan that is widely distributed and infects humans and other warm-blooded animals. Transmission to humans occurs via ingestion of oocysts shed into the environment with cat feces or through consumption of meat and meat products derived from infected animals. In individuals with healthy immune systems, *T. gondii* infection is typically asymptomatic. However, in individuals with immune systems that are compromised due to the presence of diseases such as AIDS, the parasite spreads rapidly, resulting in severe disease and encephalitis (4). *T. gondii* has been the subject of rapid investigation in recent years due to the pathologies it causes by settling in the brain tissue and nervous system (19). *T. gondii* antibody seroprevalence is around 30%, 15.8%, and 29% in Europe, America, and Turkey, respectively (20). In contrast, studies conducted especially in Central and South American countries report that *T. gondii* seroprevalence is over 60% (21). The prevalence of toxoplasmosis is associated with a number of factors, including dietary habits, age, and rural or urban living (22). Serum samples from 1,265 patients in Korea were analyzed for IgG antibody titers, and seropositivity was identified at 6.6% with the latex agglutination test and 6.7% with ELISA. The highest seroprevalence was observed in individuals aged 40-49. As documented in the clinical sciences, *T. gondii* infection rates were notably elevated among patients treated in psychiatry, ophthalmology, emergency medicine, and thoracic surgery departments (23). A study conducted in Taiwan between 2007 and 2020 yielded data on 150 domestically acquired cases of toxoplasmosis and nine imported cases. The incidence of toxoplasmosis reached its highest point in 2017, exhibiting an upward trajectory of 0.09–0.89 cases per 1,000,000 individuals. The mean annual incidence of toxoplasmosis was 4.4, 13, and 18 cases per 1,000,000 individuals during the 2007–2011, 2012–2016, and 2017–2020 periods,

respectively. A comparison of the incidence rates according to gender, age, season, and place of residence revealed that the highest rates were observed in males, patients aged 20–39, the summer months, and the eastern region. The highest rates were 1.02, 1.72, 0.38, and 3.63 cases per million population (24). It has been posited that the antibody positivity rate for *T. gondii* in women is 84%, a figure that exceeds the 32% positivity rate observed in London. This elevated prevalence has been attributed to the practice of consuming undercooked meat in France. In studies examining contamination during organ and tissue transplantation, it has been posited that this contamination may be significant due to the potential for clinical symptoms to manifest in recipients with suppressed immune systems. However, this hypothesis lacks epidemiological value (25).

Diagnosis of Toxoplasmosis in Pregnant Women

It is recommended that antibody tests for Toxoplasmosis be performed as early as possible in pregnancy. The presence of negative IgG and IgM antibodies indicates the absence of disease. However, individuals with these antibody profiles remain at risk for Toxoplasmosis if they encounter the parasite (26). In *T. gondii* disease, IgG antibodies in the first two trimesters of pregnancy indicate chronic infection. It does not pose a risk to the fetus, except for cases where the pregnant woman has a severe immune deficiency. In the third trimester, pregnant women who are IgG positive and IgM negative typically exhibit signs of a chronic infection. However, this does not rule out the possibility of an acute infection that may have occurred earlier in the pregnancy. It is currently believed that immediate medical abortion should be applied in cases where the patient is IgM-positive during pregnancy. However, it should be noted that IgM positivity does not always indicate acute infection. It is therefore recommended that these patients be referred to reference laboratories for further confirmation (27).

In individuals who were diagnosed with toxoplasma seroconversion during pregnancy, high avidity values indicate that the infection was experienced at least three to five months ago. The avidity value is a useful tool for diagnosing and treating toxoplasmosis. It shows when the infection happened, which is important for deciding if the baby will get sick. If a pregnant woman has a positive IgM value and high IgG avidity in the 14th week of pregnancy, the baby will not get infected. However, in some cases, low or suspicious IgG avidity results may not indicate a recent infection. This is because low or suspicious avidity values can persist for months (28). The utilization of avidity testing as a confirmatory test during the initial 16 weeks of pregnancy is crucial in terms of preventing the potential for high costs, the necessity for PCR examination of amniotic fluid, the administration of spiramycin to the pregnant woman, the possibility of an unnecessary abortion, and the potential for concerns within the family (28). In newborns, testing for Toxoplasma IgG antibodies

is an ineffective diagnostic tool. As IgG positivity may be of maternal origin, The antibody titers derived from the mother decrease and disappear within a period of six to twelve months. Consequently, testing for IgG antibodies is not a valuable diagnostic tool. In newborns, testing is conducted specifically for IgA and IgM antibodies. In newborns with congenital toxoplasmosis, Western blot examination is the most appropriate method of analysis, as it is capable of distinguishing between maternal and infant-derived antigens (29). The combination of Western blot examination and conventional serological tests (IgG, IgM, IgA) has been demonstrated to be an effective approach for diagnosing congenital toxoplasmosis at birth and in the initial three-month period thereafter. Furthermore, an ophthalmological examination, radiological imaging for cerebral calcifications, and a cerebrospinal fluid (CSF) examination should be conducted in all infants suspected of having congenital toxoplasmosis (30).

Relationship between Toxoplasma gondii and Schizophrenia

It has been established for a considerable period that there is a correlation between infections and psychiatric disorders. *T. gondii* infection has a high seropositivity rate across all countries globally, with an estimated 40% of the global population seropositive for *T. gondii*. This prevalence is particularly high in countries with a high consumption of sheep and goat products, such as France, England, and Austria (9). It is suggested that toxoplasmosis may significantly increase the risk of schizophrenia by interacting with host factors, such as the immune system and psychiatric predisposition, rather than being a direct cause of the disease (31). In neurological diseases such as depression, Alzheimer's, and schizophrenia observed in humans, aberrant alterations have been observed in the hippocampal region. Consequently, in experimental studies, the presence of perivascular cellular infiltration at the border of the hippocampus in chronically infected mice is regarded as a crucial factor (13,22)(32). A study of patients with schizophrenia found that those with the illness who also had a certain bacteria in their bodies were five times more likely to die than those without the bacteria. patients (33). A significant body of observational research has demonstrated a robust correlation between toxoplasmosis and schizophrenia. It is time to test this association in randomized double-blind placebo-controlled clinical trials of first-line anti-toxoplasma prophylaxis in toxoplasma seropositive patients with toxoplasmosis and schizophrenia (32). A study revealed that levels of *T. gondii* IgG antibodies and seroprevalence in schizophrenic patients were four times higher than those observed in the control group. A study found that *T. gondii* antibodies in schizophrenic patients were four times higher than in a control group. In addition, *T. gondii* IgG levels in patients exceeded 150 IU/ml, a threshold exceeded by the control group. *T. gondii* seropositivity was higher in patients who cleaned cat feces and in patients with simple schizophrenia.

A study looked at whether *T. gondii* infection in mothers increases the risk of schizophrenia and related disorders. The study found that *T. gondii* IgG antibody levels were linked to schizophrenia (34,35).

In a study conducted in Algeria, a total of 140 individuals including 70 patients affected by schizophrenia and 70 healthy controls were included. The results of this study showed that *T. gondii* seropositivity is significantly associated with schizophrenia. A study found that people under 38 with schizophrenia had higher rates of antibodies to the parasite *T. gondii* compared to people of the same age without schizophrenia. The study also suggested that schizophrenia is associated with family and parental problems (36). A study found that 25.64% of *T. gondii* seropositive cases had major depression, compared to 14.55% in the control group (37). A case report highlighted that a 32-year-old patient, who had received treatment for major depression 7 months ago but was resistant to treatment, experienced regression of depressive symptoms after receiving appropriate antiparasitic agents to treat *T. gondii* (38).

Effects of the Parasite on the Brain

In psychiatric patients with high *T. gondii* seropositivity, the risk of infection is elevated due to specific behaviors such as consuming raw meat, contact with infected cats, and lack of attention to hygiene conditions (39). *T. gondii* crosses the intestinal and placental epithelium and establishes itself in circulating macrophages and dendritic cells, to reach target organs such as the brain. In studies on mouse brains, *T. gondii* has been found to invade microglia, astrocytes, and neurons, and to develop into cysts within these cells. A similar study using human neurons and astrocytes confirmed the presence of the cystic form of the parasite within these cells (40,41). Rahdar and colleagues conducted a study on rats, showing that chronic toxoplasmosis caused an increase in dopamine levels and a decrease in serotonin levels in the brains of experimentally infected rats, which led to behavioral changes. This current study suggests that toxoplasmosis infection should be considered in human neurological diseases (42). In the case studies conducted by Brown et al with 63 patients and Mortensen et al with 71 patients with schizophrenia, blood and maternal serum samples taken from infants within a few days after birth were used (38,43). In both studies, Toxoplasma IgG and IgM antibody titers were measured. While the serum samples taken in Brown's study were of maternal origin, the serum samples in Mortensen's study were taken from newborns that were a few days old. In these samples, IgG was deemed to be of maternal origin, given that it crossed the placenta. The half-life of maternal IgG in children is one month; in contrast, *T. gondii*-specific IgG synthesis in infected newborns commences at three months of age. The presence of IgG in both studies does not indicate that the mother was infected during pregnancy; rather, it suggests that she may have been infected at some point prior to the

collection of samples (38). It is possible that anti-*T. gondii* IgGs in circulation may precipitate a pathological mechanism that is similar to the formation of some paraneoplastic central nervous system (CNS) findings. The psychiatric symptoms are correlated with the antibody levels specific to the tumor. The tumor produces proteins that are typically expressed exclusively in the brain. When these proteins are produced outside the brain tissue by the immune system, they are defined as foreign substances (44). Autoantibodies have the potential to influence consciousness, mood, behavior, and attitude. They may also contribute to pathological changes in the central nervous system (CNS), as observed in conditions such as systemic lupus erythematosus. This is thought to occur, at least in part, through binding to the N-methyl-D-aspartate (NMDA) receptor (45). A comparable potential mechanism may be contemplated in the pathogenesis of schizophrenia with anti-*T. gondii* IgG antibodies. In a seroprevalence study conducted by Tedla and colleagues, a total of 495 serum samples were evaluated for *T. gondii*, CMV, HSV-1, and HSV-2 IgG levels. The study included individuals with schizophrenia, bipolar disorder, and healthy individuals. The seroprevalence of *T. gondii* was found to be 4.7 times higher in individuals with schizophrenia and 3 times higher in individuals with bipolar disorder compared to the control group. In the same study, the researchers also observed a significant elevation in CMV IgG antibody levels in individuals diagnosed with schizophrenia and bipolar disorder when compared to the control group. Additionally, the study revealed that CMV IgG antibody levels were elevated in individuals under the age of 25, in comparison to the control group (46).

Treatment of *Toxoplasma gondii*

The treatment of toxoplasmosis is still not fully understood. However, research is focusing on six different classes of treatments. These are cytokine therapy, monoclonal antibody therapy, mesenchymal therapy, vaccine therapy, corticosteroid therapy and exosome therapy (47). Looking at them in order, cytokines are proteins produced by immune cells and are restricted by IL-12/IFN-γ. ILC1s are thought to be the source of cytokines for the treatment of cerebral toxoplasmosis. Studies have shown that IL-12 as an adjuvant enhances the immune response against the disease (48). In addition, IL-6 is effective in controlling ocular damage, IL-10, Il-27 in inhibiting allergic reactions. In studies of monoclonal antibody therapy, it has been observed that the use of IL-17A mAb and tacosides significantly suppresses parasite proliferation. Studies of IL-6 (tocilizumab), also used in autoimmune therapy, have suggested that it may be effective in treating toxoplasmosis (49). Mesenchymal therapy is another promising method due to its ease of passage through membranes. MSCs have been shown to have a parasitic effect on various parasites, including T.gondii. Corticosteroids, on the other hand, are known to cause endophthalmitis in high doses rather than being effective in treatment.

CONCLUSION AND RECOMMENDATIONS

The primary means of *T. gondii* transmission is through contact with cats. The high rate of infection in people who do not have contact with cats can be attributed to factors such as personal hygiene practices, consumption of undercooked meat, and exposure to infective forms from poorly washed vegetables and fruits. To investigate the potential causal relationship between *T. gondii* and schizophrenia, it would be necessary to conduct a population-based screening of individuals who are seropositive for *T. gondii* and monitor them for at least 5-10 years, while also evaluating them psychiatrically during this period. The undertaking of prospective cohort studies of this nature is challenging due to logistical and financial constraints. However, in regions where agriculture and animal husbandry are primary sources of income, studies on the prevalence of *T. gondii* can be especially relevant, particularly about hospital applications and the diagnosis of schizophrenia. In Turkey, it is vital to investigate T. gondii in endemic areas and not overlook this zoonotic factor in healthcare personnel's diagnoses to safeguard public health. A comparison of T. *gondii*-related risk factors in schizophrenia patients versus individuals without psychiatric health issues, alongside an assessment of the exposure of schizophrenia patients to risk factors believed to be associated with *T. gondii* transmission in comparison to a control group, can yield valuable insights. It would be beneficial to establish a causal relationship between the agent and the disease by tracking individuals who are *T. gondii* seropositive and free from psychiatric health problems prospectively. In particular, during the monitoring of pregnant women, which is easy and mandatory today, it is extremely important to investigate the presence of *T. gondii* and risk factors for the protection of regional public health. It would be advisable to perform *T. gondii* screening on cats and dogs during the same process using serological methods. It would be beneficial to record the data obtained with geographical information systems. Identifying risky areas is also important, as is monitoring individuals at risk living in these areas (primarily pregnant women, children, and individuals with psychiatric health problems). Training on healthy lifestyle behaviors, and personal and environmental hygiene could also be beneficial. To prevent the diagnosis of *T. gondii* from being missed, it might be helpful to remind healthcare personnel of their awareness and responsibility on this issue through in-service training.

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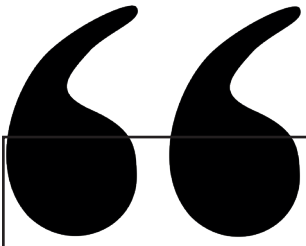

Chapter 6

AN UPDATED REVIEW ON UTERINE LEIOMYOMA REQUIRES A STRUCTURED AND DETAILED APPROACH

Selim Akkaya[1]

Teymur Bornaun[2]

Hamit Zafer Güven[3]

1 Dr. Department of Obstetrics and Gynecology, Istanbul University Health Sciences Istanbul Bagcilar Training and Research Hospital, drakkaya.selim@gmail.com, https://orcid.org/0000-0001-8344-5125

2 Op. Dr., Department of Obstetrics and Gynecology, Istanbul University Health Sciences Istanbul Bagcilar Training and Research Hospital, https://orcid.org/0009-0007-8081-8003

3 MD, Department of Obstetrics and Gynecology, Istanbul University Health Sciences Istanbul Bagcilar Training and Research Hospital, https://orcid.org/0009-002-7217-7318

1. Introduction

Uterine leiomyomas, commonly referred to as fibroids, are benign tumors predominantly composed of smooth muscle cells and fibrous connective tissue. They arise from the myometrium (muscular layer) of the uterus and are the most prevalent benign pelvic tumors in women of reproductive age. Leiomyomas vary greatly in size, number, and location within the uterus, contributing to a spectrum of clinical manifestations ranging from asymptomatic presentations to severe, life-altering symptoms such as heavy menstrual bleeding, pelvic pain, and infertility (1).

The development of uterine fibroids is believed to be influenced by multiple factors, including genetics, hormones (especially estrogen and progesterone), and growth factors that affect cellular proliferation and extracellular matrix formation. Despite their benign nature, the impact of fibroids on quality of life can be profound, necessitating significant medical attention and healthcare resources (2).

This review is critical as it addresses several key aspects of uterine leiomyomas, aiming to provide an updated understanding of their pathophysiology, diagnostic approaches, and management strategies. As the incidence of leiomyomas is high among women of childbearing age, there is a substantial interest in identifying effective conservative treatments that preserve fertility and reduce the need for invasive procedures like hysterectomy.

Furthermore, recent advancements in molecular biology and genetics have begun to shed light on the specific pathways involved in the growth and development of leiomyomas, opening new avenues for targeted therapies. This chapter will examine these advancements and discuss their implications for future research and clinical practice. It will also evaluate the current standards in surgical and non-surgical management, highlighting the role of emerging technologies and treatments in improving patient outcomes (3).

Uterine leiomyomas are estimated to affect up to 80% of women by the age of 50, although symptomatic fibroids are less common. The prevalence of these tumors varies significantly with age, racial and ethnic background, and other risk factors such as obesity and hypertension. African American women, for example, tend to develop fibroids at a younger age and with greater severity compared to their Caucasian counterparts. This review will delve into the epidemiological data, exploring how these disparities influence treatment outcomes and the burden of disease (4).

Recent genetic studies have identified several genes that are frequently mutated in leiomyomas, providing new insights into their pathogenesis. For instance, mutations in the Mediator Complex Subunit 12 (MED12) are among the most common genetic alterations found in fibroids, suggesting a crucial

role in tumor growth. This section will explore how these genetic factors contribute to the development of leiomyomas and discuss potential genetic targets for therapeutic intervention (5).

2. Background

2.1. Epidemiology

2.1.1. Incidence and Prevalence Worldwide

Uterine leiomyomas are the most common benign tumors in women of reproductive age, with studies suggesting that they affect approximately 70-80% of women by the age of 50. The incidence of clinically significant leiomyomas is slightly lower, as many cases remain asymptomatic and undiagnosed. The global burden of these tumors is significant, with large variations observed across different populations and geographical regions (6).

Epidemiological studies have consistently demonstrated that the prevalence of leiomyomas increases with age, peaking in the 40-50 year age group, just before menopause. These tumors are rare in women under 20 and their prevalence sharply declines after menopause, suggesting a strong hormonal link in their growth dynamics. Such data are crucial for health policy makers and clinicians to allocate resources and plan preventative health strategies effectively (7).

2.1.2. Demographic Variations

The incidence and severity of uterine leiomyomas vary significantly among different ethnic and racial groups. For example, African American women are known to have a higher prevalence, earlier onset, and more severe symptomatology compared to Caucasian women. Studies indicate that African American women may be up to three times more likely to develop fibroids than their Caucasian counterparts, and they also tend to experience symptoms at a younger age. This discrepancy has prompted researchers to explore genetic, socio-economic, and environmental factors that might contribute to these differences (8).

Additionally, Hispanic and Asian women also show distinctive patterns in the prevalence and impact of leiomyomas. Hispanic women report a slightly higher prevalence than Caucasians but lower than African Americans, while Asian women generally have the lowest incidence among the groups studied. These variations underscore the importance of considering ethnic and racial backgrounds when diagnosing and treating uterine leiomyomas. Tailored healthcare approaches are required to address these disparities effectively, ensuring equitable health outcomes for all affected women (9).

The role of lifestyle factors, including diet, body mass index, and physical activity, also appears to influence the development of fibroids. Obesity is

associated with an increased risk of developing fibroids, possibly due to higher levels of circulating estrogens. Conversely, physical activity has been shown to decrease this risk, suggesting that lifestyle modifications could be a vital part of managing or even preventing leiomyomas in susceptible populations (10).

2.2. Pathophysiology

2.2.1. Genetic and Molecular Mechanisms

Uterine leiomyomas are characterized by a remarkable diversity in their genetic landscape, indicating a complex interplay of multiple genetic and epigenetic factors in their pathogenesis. Several key genes have been identified that contribute to the development and growth of these tumors. Among the most significant genetic findings are mutations in the Mediator Complex Subunit 12 (MED12), which are present in up to 70% of fibroids. These mutations suggest that alterations in transcription regulation play a critical role in tumor growth (11).

In addition to MED12, abnormalities in High Mobility Group AT-hook 2 (HMGA2) gene, fumarate hydratase (FH), and collagen, type IV, alpha 5 (COL4A5) genes have been frequently observed. These genetic mutations may lead to aberrant cellular proliferation and avoidance of normal apoptotic processes. For example, leiomyomas with HMGA2 overexpression show increased levels of anti-apoptotic factors, promoting cellular survival and accumulation (12).

Epigenetic modifications also contribute significantly to the pathophysiology of leiomyomas. Changes in DNA methylation patterns and histone modifications at key gene sites affect gene expression without altering the DNA sequence, influencing tumor growth and development. These epigenetic changes can be influenced by environmental factors and may explain the variability in fibroid growth rates among different patients (13).

2.2.2. Hormonal Influences

Hormones, particularly estrogen and progesterone, play critical roles in the growth of uterine leiomyomas. Estrogen, not only stimulates the proliferation of leiomyoma cells via estrogen receptors alpha and beta but also increases the production of growth factors that can lead to fibroid growth. Progesterone, while often thought of as inhibitory, has been shown to contribute to fibroid growth by promoting the production of growth factors such as transforming growth factor-beta (TGF-β), which further stimulates fibroid cell proliferation and collagen production (14).

The estrogen and progesterone receptors are found in higher concentrations in leiomyomas than in the surrounding myometrium. This receptor overexpression enhances the sensitivity of fibroid cells to hormonal

stimulation. Additionally, the intracrine metabolism of steroids within the fibroid tissue itself can lead to localized increases in hormone levels, thus perpetuating growth in a self-sustaining environment (15).

Another important aspect of hormonal influence is the role of the extracellular matrix (ECM) in fibroid development. The ECM in fibroids is characteristically abundant and disorganized, composed predominantly of collagen and fibronectin. Hormonal influences promote the deposition of ECM components, which contributes to the fibrotic nature of the tumors and affects their mechanical properties, such as stiffness and resistance to apoptosis (16).

2.3. Clinical Manifestations

2.3.1. Symptoms and Clinical Signs

Menstrual Abnormalities: Heavy menstrual bleeding, or menorrhagia, is the most frequently reported symptom associated with uterine leiomyomas (17). This can be quantitatively assessed by the number of pads or tampons used per day, with severe cases potentially leading to anemia due to significant blood loss. Women suffering from menorrhagia may require medical intervention, including iron supplementation or more aggressive treatments such as hormonal therapy to manage the bleeding and prevent anemia (18).

Pelvic Pressure and Pain: Fibroids can vary greatly in size, and as they grow, they can cause discomfort or pain due to pressure on nearby organs. This can manifest as a constant dull ache or periodic sharp pains, especially during periods. Large fibroids may also contribute to pain during intercourse, a symptom that significantly affects the quality of life and emotional well-being of patients (19).

Bulk Symptoms: The mass effect of substantial fibroids can mimic the symptoms of pregnancy, including increased abdominal girth and urinary frequency. These symptoms can lead to misdiagnosis and inappropriate management if not correctly attributed to fibroids (20).

Reproductive Issues: The impact of fibroids on fertility includes altered endometrial receptivity, mechanical obstruction of gamete transport, and distorted uterine anatomy, all of which can impair implantation and increase the risk of miscarriage (21). Case studies have highlighted the challenges in managing pregnancies complicated by fibroids, which may involve decisions regarding the timing of fibroid removal and the mode of delivery (22).

2.3.2. Complications Associated with Leiomyomas

Acute Pain and Torsion: Pedunculated fibroids are prone to torsion, which can cause acute, severe abdominal pain and is considered a surgical emergency. This complication requires immediate evaluation and intervention

to prevent further complications such as necrosis or infection (23).

Degeneration: Fibroid degeneration, particularly red degeneration, can occur during pregnancy due to rapid growth and outstripping of blood supply. This condition is associated with acute pain and may be mistaken for other obstetric emergencies such as placental abruption or preterm labor (24).

Infertility and Pregnancy Complications: Fibroids are associated with a range of reproductive issues, from infertility to recurrent miscarriages. Moreover, they can lead to complications during pregnancy such as increased cesarean section rates, fetal growth restriction, and postpartum hemorrhage (25).

Heavy Menstrual Bleeding and Anemia: Chronic heavy menstrual bleeding can lead to significant iron-deficiency anemia, which may require not only medical and surgical therapies but also psychosocial support due to the impact on the patient's quality of life (26).

Urinary and Bowel Problems: Compression of the bladder and intestines can lead to frequent urination, urgency, and constipation. In severe cases, fibroids can cause hydronephrosis by obstructing the ureters, which might require surgical intervention to relieve the obstruction and prevent kidney damage (27).

2.3.3. Psychosocial and Quality of Life Impacts

The presence of fibroids can severely impact a woman's quality of life. Chronic pain, heavy menstrual bleeding, and the associated symptoms can lead to significant psychological distress. Issues such as anxiety, depression, and body image disturbances are common among women dealing with the symptomatic fibroids. Social stigma and misconceptions about fibroids also contribute to emotional distress (28).

Comprehensive management strategies, including patient education, psychological support, and tailored treatment plans, are essential for improving the overall well-being of women affected by uterine leiomyomas.

2.4. Diagnosis

The diagnosis of uterine leiomyomas is predominantly based on clinical symptoms and imaging studies, with biomarkers playing a supportive role in both diagnosis and monitoring treatment responses.

2.4.1. Imaging Techniques

Ultrasound: Transvaginal ultrasound (TVUS) is the primary imaging technique used for the initial assessment of uterine leiomyomas. It provides detailed information about the size, number, and location of fibroids within the uterine wall. TVUS is highly accessible, cost-effective, and capable of

differentiating fibroids from other gynecological abnormalities such as ovarian tumors or endometrial polyps (29). The technique is particularly useful for planning surgical interventions and assessing potential impacts on fertility.

Magnetic Resonance Imaging (MRI): MRI is a more sophisticated imaging modality that offers superior resolution and better delineation of fibroids compared to ultrasound. It is especially useful in the preoperative assessment of patients with multiple or very large fibroids, helping to map the fibroids in relation to other pelvic organs and blood supply. MRI can provide valuable information on the characteristics of fibroids, such as their potential for malignancy or the likelihood of certain complications like degeneration (30).

Hysterosalpingography (HSG): While primarily used in the evaluation of the uterine cavity and fallopian tube patency in infertility assessments, HSG can also help identify submucosal fibroids that distort the uterine cavity. This method involves the injection of a radiopaque dye into the uterine cavity, followed by X-ray imaging. HSG is particularly valuable for planning fertility-preserving treatments (31).

Computed Tomography (CT): Although less commonly used due to its lower sensitivity and higher radiation exposure compared to MRI, CT can be employed in certain cases where other imaging modalities are inconclusive or unavailable. CT scans can help detect calcified fibroids and assess the impact of large fibroids on other abdominal organs (32).

2.4.2. Biomarkers and Laboratory Tests

While there are no specific serum biomarkers for the diagnosis of uterine leiomyomas, several laboratory tests can be useful in the evaluation and management of associated symptoms:

Hemoglobin and Hematocrit Levels: Given that heavy menstrual bleeding is a common symptom of fibroids, assessing hemoglobin and hematocrit levels is crucial for determining the presence and severity of anemia. Chronic blood loss can lead to iron deficiency anemia, necessitating appropriate management to improve the patient's quality of life and functional status (33).

Hormone Levels: Although not diagnostic, measuring serum levels of estrogen and progesterone can provide insights into the hormonal milieu that may be contributing to fibroid growth. These measurements are particularly relevant in patients considering hormonal therapy as part of their fibroid management strategy (34).

Cancer Antigen 125 (CA-125): Elevated CA-125 levels can be associated with a wide range of gynecological conditions, including fibroids, though it is not specific. It may be elevated in cases where fibroids lead to significant pelvic inflammation or in cases of concurrent conditions such as endometriosis. While not diagnostic for fibroids, it can be a useful marker for tracking treatment response, especially in those undergoing medical therapies (35).

Newer Biomarkers: Research into specific biomarkers for fibroids is ongoing, with several potential candidates under investigation. These include growth factors, cytokines, and other cell-signaling molecules that play roles in fibroid pathophysiology. For instance, transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) have been found in higher concentrations in women with fibroids and may eventually serve as both diagnostic and therapeutic targets (36).

2.5.Management and Treatment

2.5.1. Medical Treatments

Hormonal Therapies: These are among the first-line treatments for reducing fibroid symptoms, especially abnormal uterine bleeding. Hormonal treatments include the use of oral contraceptives, which can help regulate menstrual cycles and reduce menstrual flow. Gonadotropin-releasing hormone (GnRH) agonists are another option, effectively shrinking fibroids by creating a temporary menopausal state and decreasing estrogen production. However, due to their side effects, such as bone density loss, their use is generally limited to short-term treatment before surgery (37).

Progesterone Receptor Modulators: Drugs like ulipristal acetate can reduce fibroid size and control bleeding. They act by modulating the progesterone receptors on the fibroids, inhibiting their growth and reducing symptoms. These medications can be an alternative to surgery for some women and are useful in managing symptoms preoperatively (38).

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): While NSAIDs do not reduce fibroid size, they are effective in managing the pain associated with fibroids. They are often recommended during menstrual periods when pain and bleeding are at their peak (39).

Iron Supplements: For women experiencing significant menstrual bleeding leading to anemia, iron supplements are crucial to manage the resultant iron deficiency. This is a supportive treatment that addresses the symptoms rather than the fibroids themselves (40).

New Pharmacological Treatments: Research into drugs that can specifically target the biological mechanisms of fibroids is ongoing. This includes novel agents that inhibit growth factors or disrupt the fibroid's

vascular supply, aiming to reduce fibroid size and symptoms with fewer side effects than current therapies (41).

2.5.2. Surgical Options

Myomectomy: This is the surgical removal of fibroids while preserving the uterus, making it the treatment of choice for women who wish to maintain fertility. Myomectomy can be performed using various techniques, including hysteroscopic, laparoscopic, or open surgery, depending on the size, number, and location of the fibroids (42).

Hysterectomy: The surgical removal of the uterus provides a definitive solution for fibroids. It is typically considered when other treatments have failed, fibroids are extremely large, or when the woman does not wish to preserve her fertility. Hysterectomy is the only permanent cure for fibroids but it also ends a woman's ability to bear children and may have long-term hormonal consequences (43).

Uterine Artery Embolization (UAE): This minimally invasive procedure involves cutting off the blood supply to the fibroids by injecting small particles into the uterine arteries. UAE is highly effective in shrinking fibroids and alleviating symptoms. It offers a less invasive alternative to surgery with a shorter recovery time and is an option for women who are not candidates for surgery or who wish to avoid hysterectomy (44).

MRI-guided Focused Ultrasound Surgery (MRgFUS): This is a non-invasive, outpatient procedure that uses high-frequency ultrasound waves to heat and destroy fibroid tissue. It is guided by magnetic resonance imaging (MRI) to precisely target the fibroids while sparing surrounding healthy tissue. MRgFUS is suitable for treating selected fibroids and offers the advantage of being completely incision-free with minimal recovery time (45).

Endometrial Ablation: Although not a primary treatment for fibroids, endometrial ablation can be effective in reducing menstrual bleeding in cases where fibroids are small and submucosal. This procedure involves destroying the lining of the uterus (endometrium) and is only suitable for women who do not wish to have children in the future (46).

2.5.3. Emerging Therapies

The landscape of uterine fibroid treatment is continually evolving with the development of new therapeutic options that aim to offer efficacy while minimizing side effects and preserving fertility.

Selective Progesterone Receptor Modulators (SPRMs): Building on the success of earlier progesterone modulators, newer SPRMs are being developed to offer more targeted therapy with fewer side effects. These drugs aim to reduce fibroid size and control bleeding while minimizing impact on

the endometrium, thus preserving fertility and reducing the need for surgical intervention (47).

Gonadotropin-Releasing Hormone (GnRH) Antagonists: Unlike GnRH agonists, which initially cause a surge in hormone levels before suppression, GnRH antagonists provide immediate suppression of hormone levels. This results in a rapid reduction in fibroid size and symptoms, making them a valuable option for preoperative treatment or for women who cannot undergo surgery (48).

Vitamin D and Green Tea Extract (Epigallocatechin gallate - EGCG): Recent studies suggest that vitamin D and components found in green tea may inhibit fibroid growth. These natural compounds could provide a non-invasive treatment alternative or complement existing therapies, especially for patients with mild symptoms or those who prefer to avoid conventional medical treatments (49).

Anti-Angiogenic Drugs: Given that fibroids require an extensive blood supply to grow, targeting angiogenesis (the formation of new blood vessels) represents a promising therapeutic approach. Drugs that inhibit vascular endothelial growth factor (VEGF) and other angiogenic factors are under investigation for their potential to starve fibroids and induce regression (50).

2.5.4. Prognosis and Outcomes

Most women with uterine fibroids experience significant improvement in their symptoms following appropriate therapy, whether medical, surgical, or through emerging treatments. The choice of treatment largely depends on the severity of symptoms, size and location of the fibroids, desire for fertility preservation, and patient preferences (49-51):

Fertility and Pregnancy Outcomes: For women who undergo conservative treatment such as myomectomy, the prognosis for fertility is generally good. However, the presence of multiple fibroids, particularly those distorting the uterine cavity, can pose challenges. Post-treatment, many women are able to conceive, although they may be more likely to require cesarean delivery depending on the residual effects on the uterine structure.

Long-Term Health: After treatment, especially surgical, most women see a permanent resolution of symptoms such as heavy bleeding and pelvic pain. However, hysterectomy, while curative for fibroids, can lead to long-term hormonal and metabolic changes if the ovaries are also removed. Therefore, the long-term prognosis must consider potential menopausal symptoms and cardiovascular health implications.

Recurrence: Fibroids can recur after treatment, especially if the ovaries are preserved and hormonal stimulation continues. The risk of recurrence is

particularly noted in younger women and those with multiple fibroids at the initial diagnosis. Regular follow-up with imaging studies is recommended for those at high risk of recurrence to manage new fibroids effectively before they cause significant symptoms.

Quality of Life: The overall quality of life for patients treated for uterine fibroids typically improves significantly post-treatment. Reduction in pain, normalization of menstrual cycles, and the resolution of anemia and other associated symptoms contribute to improved general health and well-being.

2.6. Factors Influencing Prognosis

The prognosis for women with uterine fibroids varies based on several factors, which can affect the choice and effectiveness of treatment strategies (48-50):

Age and Menopausal Status: Younger women tend to have more aggressive fibroid growth due to higher hormone levels, and thus may face a higher risk of recurrence after treatment. Postmenopausal women generally experience a natural reduction in fibroid size and symptoms as estrogen levels decline.

Fibroid Size, Number, and Location: Larger and more numerous fibroids, or those located within the uterine cavity (submucosal), often result in more severe symptoms and may be more challenging to treat conservatively. These factors can also influence surgical complexity and the risk of complications such as excessive bleeding or damage to the uterus during removal.

Reproductive Plans: The desire to maintain fertility is a significant factor influencing treatment choices and outcomes. Women wishing to conceive may opt for myomectomy or other fertility-preserving treatments, which may have variable success rates depending on the fibroids' characteristics and any resulting uterine damage or scarring.

Genetic and Molecular Factors: Emerging research suggests that specific genetic profiles may predict fibroid growth patterns, treatment response, and recurrence risks. Understanding these profiles can help tailor treatment plans and anticipate prognosis more accurately.

Overall Health and Comorbidities: General health status, including the presence of coexisting conditions such as diabetes or hypertension, can impact the management of fibroids. These conditions may affect surgical risk, choice of anesthesia, and potential for complications during and after treatment.

2.6.1. Long-term Management

Managing uterine fibroids effectively over the long term involves a combination of regular monitoring, lifestyle adjustments, and sometimes ongoing treatment (51-53):

Regular Medical Follow-Up: After initial treatment, regular follow-up appointments with imaging tests like ultrasound or MRI are crucial to monitor for new fibroid growth or recurrence. This is especially important for women who retain their reproductive organs and those who are on medications like GnRH agonists or SPRMs, which may only provide temporary relief.

Lifestyle Modifications: Dietary and lifestyle changes can help manage fibroid symptoms and overall well-being. A diet rich in fruits, vegetables, and whole grains, low in red meat, and moderate in dairy has been suggested to reduce fibroid risk or growth. Regular exercise can help manage weight, reduce hypertension, and improve cardiovascular health, potentially influencing fibroid growth indirectly.

Hormonal Management: For some women, long-term management may include ongoing hormonal treatments to control fibroid growth and symptoms. This might involve hormonal contraceptives, progesterone receptor modulators, or other therapies aimed at stabilizing the endometrial lining and reducing menstrual blood loss.

Alternative and Complementary Therapies: Some patients find relief in alternative therapies such as acupuncture, herbal treatments, or yoga. These methods can help manage symptoms, particularly pain and stress, which are often associated with fibroids. However, patients should discuss these approaches with their healthcare provider to ensure they are safe and complementary to standard medical treatments.

Psychological Support: Given the potential impact of fibroids on quality of life and mental health, psychological support or counseling can be beneficial for some women. Support groups or therapy can help manage the emotional and psychological effects of living with a chronic condition like uterine fibroids.

3. Conclusion

Research in uterine leiomyomas is rapidly advancing across multiple fronts, from understanding the molecular and genetic basis of fibroids to developing innovative therapeutic approaches:

Genetic and Molecular Research: One of the most promising areas of fibroid research involves unraveling the genetic alterations associated with fibroid development. Studies are increasingly focusing on the role of non-coding RNAs, such as microRNAs and long non-coding RNAs, which regulate gene expression at the post-transcriptional level. These molecules are crucial for controlling cell proliferation and apoptosis, and their dysregulation may contribute to fibroid pathogenesis. Identifying specific microRNAs associated with fibroid growth offers potential targets for novel therapies that could selectively inhibit fibroid cells without affecting the surrounding uterine

tissue.

Personalized Medicine: As the understanding of the genetic makeup of fibroids improves, there is a growing interest in personalized medicine approaches. These strategies involve tailoring treatments based on the individual genetic profile of a patient's fibroids. This could lead to more effective and less invasive treatments, reducing the need for surgery and improving fertility outcomes for women affected by fibroids.

Improved Imaging Techniques: Advanced imaging technologies are being developed to enhance the diagnosis and monitoring of fibroids. High-resolution ultrasound and innovative MRI techniques, such as diffusion-weighted imaging, are being studied for their ability to provide more detailed information about fibroid size, location, and response to treatment. These advancements could lead to earlier detection and more precise mapping of fibroids, facilitating better treatment planning.

Pharmacological Advances: The development of new pharmacological agents continues to be a significant focus of fibroid research. Drugs that target specific pathways involved in fibroid growth, such as growth factor inhibitors, anti-angiogenic agents, and selective progesterone receptor modulators (SPRMs), are under investigation. These therapies aim to provide effective symptom relief while minimizing side effects and preserving fertility.

Minimally Invasive Therapies: Research is also focusing on refining and expanding minimally invasive therapeutic options. Techniques such as radiofrequency ablation, cryoablation, and focused ultrasound surgery are being studied for their effectiveness in treating fibroids with less recovery time and fewer complications than traditional surgery. These methods hold promise for improving patient comfort and satisfaction while maintaining treatment efficacy.

Stem Cell Research: Emerging evidence suggests that stem cells may play a role in the development and regeneration of fibroid tissues. Understanding the stem cell dynamics in the uterine environment could open new avenues for treatments that target the fundamental processes of fibroid formation and growth, potentially leading to more durable therapeutic outcomes.

Looking ahead, the field of fibroid research is poised to capitalize on these trends with several key objectives:

1. Integration of Multi-Omics Data: By integrating genomic, transcriptomic, proteomic, and metabolomic data, researchers aim to develop a comprehensive understanding of fibroids that can lead to breakthroughs in prevention and treatment.

2. **Enhancing Patient-Centric Outcomes:** Future research will likely focus more on patient-centric outcomes, including quality of life, fertility preservation, and symptom management, tailoring treatments to meet the specific needs and preferences of individual patients.

3. **Collaborative and Interdisciplinary Approaches:** Collaborations across disciplines such as genetics, molecular biology, pharmacology, and gynecology are crucial for driving innovations in fibroid treatment. These collaborative efforts can accelerate the translation of research findings into clinical practice.

4. **Global Health Initiatives:** Given the significant burden of fibroids, particularly in underserved populations, future research will also need to address global health disparities. This includes developing cost-effective, accessible treatment options and educational programs to increase awareness and early detection.

Potential Areas for Future Investigation

- **Targeted Drug Delivery Systems:** Developing drug delivery systems that specifically target fibroid tissues could dramatically increase treatment efficacy while minimizing side effects. Researchers are exploring the use of nanoparticles and liposomes to deliver therapeutic agents directly to fibroids. This approach could enhance the concentration of the drug in the fibroid while sparing healthy tissues, potentially reducing the systemic side effects commonly associated with fibroid treatments.

- **Immunotherapy for Fibroids:** The immune landscape within the uterine environment plays a significant role in fibroid growth and symptoms. Investigating the role of immune cells and cytokines in fibroid pathology could open new doors for treatments. Immunotherapy approaches that modify the immune response to fibroid cells could be developed, aiming to reduce fibroid growth or induce fibroid regression.

- **Genetic Editing Techniques:** With advances in genetic technologies like CRISPR/Cas9, future research might explore the possibility of correcting genetic mutations associated with fibroid development directly at the DNA level. This approach holds promise for a permanent cure for fibroids, particularly for those with a strong genetic predisposition.

- **Fibroid Stem Cell Biology:** Identifying and characterizing the stem cells that may give rise to fibroids could provide insights into how these tumors initiate and progress. Targeting fibroid stem cells could lead to novel treatments that prevent fibroid formation from the outset rather than managing the symptoms after they have developed.

- **Role of the Extracellular Matrix (ECM):** The ECM plays a critical role in the growth and maintenance of fibroids. Research into the specific components of the ECM in fibroids, such as collagen and fibronectin, and their interactions with fibroid cells could lead to therapies that disrupt these interactions and inhibit fibroid growth.

- **Microenvironment and Vascular Changes:** Understanding the changes in the uterine microenvironment and vascular structure that accompany fibroid growth could lead to interventions that target these specific changes. For example, therapies that alter blood flow to fibroids or modify the local hormonal environment could effectively reduce fibroid size and symptoms.

- **Lifestyle and Environmental Factors:** More studies are needed to clarify the connections between lifestyle factors, such as diet and exercise, and the risk of developing fibroids. Additionally, investigating environmental exposures that may increase fibroid risk could lead to public health strategies to prevent fibroid development in susceptible populations.

- **Fibroids in Menopause:** Despite the general reduction in fibroid symptoms after menopause, some postmenopausal women continue to experience fibroid-related problems. Future research could explore why some fibroids persist or continue to cause symptoms post-menopause and how these can be effectively managed.

- **Psychosocial and Economic Impacts:** There is a need for further research into the psychosocial and economic impacts of fibroids on women's lives, including work absenteeism and healthcare utilization. This research could inform policy changes and support services to better address the needs of women with fibroids.

- **Global Health Disparities:** Given the significant disparities in the prevalence and impact of fibroids among different ethnic and socioeconomic groups, future investigations should focus on understanding and addressing these disparities. This includes developing culturally tailored educational programs and healthcare interventions that improve access to effective fibroid treatment worldwide.

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Chapter 7

INVESTIGATION OF THE EFFECT OF PHOSPHOLEVODOPA ON THE TREATMENT AND QUALITY OF LIFE OF PARKINSON'S PATIENTS

Arslan SAY[1]

1 Lecturer, Amasya University, Sabuncuoğlu Şerefeddin Vocational School of Health Services, Amasya, TURKEY, Email: arslan.say@amasya.edu.tr ORCID: 0000-0001-5454-3105

1. Introduction

In the last few years of the last century, L-dopa, a simple molecule, changed the way we view Parkinson's disease (PD) and the way we treat it. But, as there is no "new L-dopa," one of the areas of research today investigates the quantitative and kinetic variations that must lead to an overwhelming evolution of it. So, the scenario is that many laboratories keep "cooking" L-dopa considering some updated physical organic chemistry in the aim to overcome its limits. In this essay, we will denote one of these attempts, because we want to evaluate if and to what extent this new molecule, named phospholevodopa, and its subcutaneous administration through pumps has the potential to modify the treatment and the quality of life (QOL) of the patient, as well as the therapeutic context. In 2016, a panel of ten Italian specialists concluded that although L-dopa is well tolerated for the ultra-majority of PD patients, some motor and/or non-motor effects could be considered disabling and require a modification of treatment. As a result, the patient's overall treatment satisfaction and his QOL were reported as moderate or insufficient by approximately 20% and 50% of cases, respectively. A scenario that could alter if improved formulations based on L-dopa were available (Agid et all., 1993; Gelb et all., 1993).

The pivotal role of L-dopa in the symptomatic treatment of PD is conventionally well established. However, his doctor's bag appears full of opportunities and risks extrapolating it. Studies on L-dopa currently in phase 3 of clinical development are mostly time-evaluated hypodermic injectables or intended and/or obtained in combination with other molecules. In 2005 in this laboratory, it was reported that a L-dopa alkyl ester shows, both in vivo and in vitro, an in vitro metabolic half-life that is lengthened by up to 15-fold. This report stems precisely from the possibility of an in vivo prolonged release of L-dopa decided to study the possible clinical activities of this new L-alpha-alkyl-phenylalanine ethyl ester chloride, named phospholevodopa, as well as its adverse reactions. In recent hours, reports have suggested the involvement of esterase's in abnormal Alpha-synuclein and acetic acid mill software.

1.1. Background of Parkinson's Disease

Parkinson's disease (PD) is the second most frequent neurodegenerative disease, after Alzheimer's disease, and is associated with a decrease in life expectancy. It is characterized by decreased mobility and the risk of complications for bedridden patients. The biological mechanism of PD is complex and not fully understood. The phenotypic evolution of the disease varies due to irreversible degenerative damage to different brainstem nuclei, such as the locus niger, pedunculopontine nucleus, and cholinergic nuclei. Motor symptoms are just the tip of the iceberg in a wide spectrum of non-motor symptoms that dominate as the disease progresses and significantly impact the quality of life of patients (Coelho et all., 2012).

In this evolving scenario, patients play a particularly important role, making it difficult to accurately assess the impact of the disease and the therapeutic effect, especially in patients over 55 years old, and whether they are in the early or advanced stages of the disease. Society has now accepted the psychological impact of the diagnosis and the appearance of these symptoms, which were previously demonized for obvious reasons related to their ethology (Olanov et all., 2009).

Despite significant advances in the development of new treatments for PD in recent years, many patients are still limited in accessing drug therapy. The fluctuating effects of the drugs also make it difficult for patients to leave their homes due to fear and embarrassment. The availability and discontinuity of the prescribed drugs, rather than the choice of drugs available in different healthcare facilities, have led to the use of continuous isoflavone. This has resulted in the development of new treatment protocols, including advanced surgical and non-surgical techniques for various routes of drug administration (such as myocardial and subcutaneous), as well as invasive techniques and clinical studies. These advancements have increased the frequency and depth of physio pathological issues observed in this challenging field.

The evaluation of new methods for delivering levodopa, such as the use of an alcoholic Parkinson pump, has already played a significant role in finding potential solutions for PD treatment (Antonini et all., 2016).

1.2. Current Treatment Modalities

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder. It is characterized by the development of a constellation of motor and non-motor activities. There is currently no substantial survival in PD patients until we find a substitute or complementary treatment regimen. The current treatment modalities in PD are usually predominantly symptom-based and are post-diagnostic processes. Physical and speech therapies, as well as surgical and pharmacological treatments, are recognized as treatment modalities. Even though there are various treatment modalities, physical, speech, and occupational therapies have little evidence. As a result, the principal methods for the management of PD in clinical practice are medications as they have vast and good research in this area. Glutamate antagonists, antioxidants, growth factors, opiates, GABA agonists, opiates adjuvant, cannabinoids, and glutamate agonists are predominantly used for clinical practice of the disease. A combination of several drugs is often prescribed to help control the symptoms, which improve the quality of life of people with PD. Caregivers can use a number of surgical techniques for PD, such as deep brain stimulation, to address some of these therapeutic challenges (Jankovic and Aguilar, 2008).

Generally, PD is treated with conservative (dopamine replacement therapy, deep brain stimulation), complementary (physical and speech therapy), and surgical (pallidotomy, thalamotomy) management. Since there is no cure for PD, most management modalities are focused more on pharmacological therapy. Dopamine agonists, monoamine oxidase type B inhibitors, catechol-O-methyltransferase inhibitors, anticholinergics, and amantadine are generally used for PD treatment purposes; it is evident that these drugs mainly influence the dopamine level. Since these therapies often wear off or stop working for some PD people, they opt for deep brain surgery or Duadap. Hundreds of trials are being conducted to evaluate the effectiveness of these therapeutic strategies and to fill in evidence gaps on the comparative benefits and harms of available drug and surgical options. Even though the drugs are available and used for PD treatment, there is a challenge in which the treatment cannot provide a therapeutic effect continuously (on-off problems and motor diseases re-emerged with side effects on the drug) despite the medication and deep brain stimulation surgeries. This is a significant challenge, as there is no treatment for the disease in many countries in sub-Saharan Africa and other densely poor areas. Thus, exhibiting a novel innovative therapeutic strategy for PD is particularly in demand. An understanding of the current therapies as shown above will help researchers understand the evidence gap, and thus this evidence will help recognize the unmet needs of the researchers regarding PD treatment. Thus, an understanding of the previous section will help researchers understand how our proof will guide other innovative suggestions to deal with these queries (Hasan et all., 2023).

2. Phospholevodopa: Mechanism of Action and Pharmacokinetics

Phospholevodopa is an analogue with a high structural resemblance to l-DOPA. It is a vitamin B6-dependent decarboxylase inhibitor (DDI), preventing the peripheral metabolism of l-DOPA through decarboxylation. Phospholevodopa diffuses more easily through the blood-brain barrier (BBB) than l-DOPA because of its lipophilic moiety, the phosphonate group. Once in the CNS, the compound is hydrolysed to l-DOPA, and the beneficial characteristics of l-DOPA are thought not to be influenced by the high connectivity to the plasma protein (Fox et all., 2008).

The first pharmacokinetic data of this medication were recently published because of the commercial interest of an international concern where also Belgian patients were in. The data of this 'open' trial were considered ideal for our goal. Knowledge about the behaviour of phospholevodopa in the body is indeed beneficial both for the assessment of the potential therapeutic impact of the drug as for the comparison with 'normal' medication and possibly revealing clues about the working mechanism (Müller, 2011). When phospholevodopa is administered as a tablet, the measure of its low bioavailability is due to a first-pass effect. Also, the rapid 'dying out', with a half-life of around 1 h, could be

explained by photochemical degradation of the compound in the blood. The latent accumulative phenomenon in steady state could indicate systemic slow-release pharmacokinetic properties. The last probably underlies the possible advantage of long-acting delivery. The percutaneous system could be rapidly placed and removed, avoiding surgical complications, and continuously slowly deliver a high level of the compound (Baláž et all., 2023).

2.1. Pharmacological Properties of Phospholevodopa

Phospholevodopa (PL-Dopa) displays physical properties like those of its parent compound, levodopa, because an almost equimolar mixture of the diastereomers 30 and 60 of PL-Dopa consequently influences the similarity of its physicochemical properties to the pure L- and D-threo-L-Dopa isomers. The main differences between the two diastereoisomers of PL-Dopa at the pharmacodynamic level are different interactions with several types of receptors. For example, unlike the L-form, D-threo-PL-Dopa interacts more energetically with the D2 type of dopaminergic receptor, and the opposite is seen in the case of adrenoreceptors.

In addition, differences in the biotransformation of exogenous PL-Dopa dosages were directly demonstrated at the pharmacokinetic levels. The most important difference in the pharmacokinetics is the contribution of two isomers to the generation of a phenolic biotransformation product, such as the catecholamine D-threoisomer of 3-O-methyldopa and the D-threoisomer of phospho-decarboxylated levodopa. In conclusion, the interaction of the Fischer addition products with the Dopa-enkephalin complex can reasonably be predicted from the interaction of phospholevodopa with the carboxymethyl-levodopaate (CML-Dopa) complex. The results obtained with these two electrochemical methods showed the typical voltammetric characteristics of phospholevodopa (Baláž et all., 2023). In fact, phospholevodopa presents a couple of reversible oxidation and reduction peaks in the medium of an H2SO4 solution. The reversible electrochemical behaviour of phospholevodopa is typical of the formation of a stable complex between the phosphate-water and the enol-form of levodopa (Rosebraugh et all., 2021). The acute toxicities of putative dopaminergic compounds have been related to their behaviour towards the lipid-protein compartment. In the present experiments, we therefore studied the effects of 0.5 M Na+ salts of L-threo-isomer of 3-phospho-dopamine (GBPxionyl), 30 phospho-D-threo-levo-dopamine (PL-Dopa), 60 DL-threo-3,4-dihydroxyphenylserine, and dopamine on the antioxidative defensive system (ADES) and the concentration of free forms of the brain neurotransmitters GABA, glutamate, and acetylcholine. The potential activating effects of GBPx on ADES will also be discussed in terms of a possible relationship with the mechanism of motor muscle control. The differences between GBPx and L-Dopa or PL-Dopa on the concentration of the neurotransmitters are also discussed in terms of the possible protein-binding

way of the diphosphate neurodrugs. In conclusion, PL-Dopa seems to have peculiar pharmacological properties, regardless of the carboxylation status, able to be of eventual clinical significance (Sletzinger et all., 1963; Nyholm et all., 2020).

2.2. Comparison with Levodopa

Phospholevodopa (PLD) is a potential antiparkinsonian drug that is converted to levo-3,4-dihydroxyphenylalanine (L-DOPA) after oral administration following liver uptake. The degradation to the inactive metabolites N-methyl-4-phenylpyridinium (MPP+) and formaldehyde is delayed, so that the resulting elevation of the L-DOPA concentration protects dopaminergic neurons of the substantia nigra. After placement in the medial forebrain bundle of 6-hydroxydopamine (6-OHDA) treated rats, four times higher striatal DOPAC values as a parameter for the dopaminergic turnover were observed compared to animals treated with L-DOPA, which was the only reduced metabolite.

A comparison with levodopa, which is the gold standard in the treatment of Parkinson's disease, is very interesting. After oral administration of phospholevodopa, a similar behaviour of the ratio of L-DOPA vs. DOPAC concerning levodopa was observed with about one half of the DOPA content and, thus, a very low fraction of phospholevodopa in human disease as well was observed. Owing to the neuroprotective capacity of phospholevodopa, a higher dosage as well as a more frequent administration (pump-controlled) than the standard treatment with levodopa should, for obvious reasons, still be the subject of further research. With up to 60 years of Parkinson's disease treatment with DOPA consisting of up to 4 doses of *Madopar,* we will soon be able to answer this question. Conversely, it is to be hoped that in the future even better quality of life as well as a cure for Parkinson's disease will be achieved (Sako et all., 2023).

3. Subcutaneous Administration of Phospholevodopa

The most specialized method of the L-amino acid prodrug of levodopa (LAAPD) called phospholevodopa (PL-PGP) delivery is the subcutaneous administration using an infusion pump. Subcutaneously, PL-PGP gets transferred to the extracellular space for metabolism through dopa-decarboxylase localized in the aromatic L-amino acid decarboxylase round some cells for central nervous system. In the standard program and the alternative 10 and 20% infusion rates, the subcutaneous administration pump treatments reach a fast levodopa plasma peak too (20 minutes) with not high Cmax plasma level, guaranteeing an easier use of very higher dosage of PL-PG compared by 2 parameters: plasmatic peak and equal AUC with more dis-longing of treatment (Baláž et all., 2023).

The subcutaneous administration of carbidopa-levodopa and entacapone can be done by 3 infusion rates, the 70-30% standard rate, the 115% pump flow rate and the 130% pump flow rate. Analysing the three different infusion rates during a morning time with any oral medication (from 7:00 a.m. to 12:30 p.m.), it was checked that higher-plasmatic levodopa peaks came in with the lower flow rate, and the longer the treatment was held the greater the difference was. To conclude, the subcutaneous administration of levodopa/pl-levodopa is a valid treatment in testing for PD patients and one treatment to consider for this chronic disease. However, it does require greater awareness due to the risk of thrombosis, as well as the importance of the treatment to be coached through a pump. The subcutaneous route of administration can be saved also to administer levodopa as a rescue therapy once the response to the continuous stimulation has faded. We used PL-PGP in our studies both plasmas intravenously and administrated subcutaneously using continuous infusion by a pump any to PM and PDLP patients (Cardinal-david et all., 2016).

3.1. Advantages and Challenges

Subcutaneously administered phospholevodopa offers several advantages over orally administered levodopa, one of the main ones being a major decrease in Cmax. This is advantageous because: a lower peak means less pulsatile stimulation of dopaminergic receptors (or, in other words, a decrease in the "on-off" phenomenon); a decrease in peak and Cmax reduces the symptoms that are effectively worse by higher levodopa systemic levels (supranuclear dyskinesias); oral phospholevodopa must be given in a fasting condition while subcutaneous can be given in any condition and combined with other meals especially in the ND treatment schedule. There are also potential challenges associated with subcutaneous administration of levodopa. One is the fact that it mandates using an implantable pump which is not always well accepted by patients. In addition, one cannot deny, as much as doctors may like the idea, that it requires a certain depth of injection, and thus can be a drawback for patients with a high fear of the needle.

Withdrawal when no alternative is available is indeed a major issue with the use of DUODOPA as a last-line treatment for so-called complex-advanced PD. Abrupt clinical decline and dopamine withdrawal syndromes are described associated with Duodopa treatment. In these cases, clinical worsening occurs within days, with or without hyperthermia or mental confusion, and all patients suffering from it would have required a morphine pump. Would phospholevodopa injections deliver the same dopamine withdrawal syndrome? There is no reason to expect this would not happen. Instead, duodenal withdrawal may be more "disastrous" because the ratio of Cmax is less affected by dopa decarboxylase inhibitor (DDCI) administration: 10% after single enteral dose vs. 19% during the interdose interval from the

implantable pump, causing a 0.3 ng/ml and 2.8 ng/ml decrease from plasma glucose, respectively, if administered in a fluid/meal bolus (Baláž et all., 2023).

4. Pumps for Drug Delivery in Parkinson's Disease

The continuous or precise administration of medication is of importance in various disease states, including Parkinson's disease, where (oral) drug treatment options may be limited. Over the years, pumps have been used with subcutaneous drug delivery routes as an alternative to oral drug delivery and treatments/diseases like rapidly progressing Parkinson and cancer (pain). Many other potential applications, such as in obsessive-compulsive disorder, Huntington's disease, epilepsy, and psychiatric diseases, are under discussion. A Dutch guideline and consensus for pump treatments have been written, and there are several articles about (safety and) port/pump placement, maintenance, and preparation of pump solutions. Subcutaneously administered drugs in general are water-soluble, therefore liposolubility is no issue in the selection of therapeutic agents. In this article, we will describe the features and pitfalls of pumps, starting with the background of drug delivery in Parkinson's disease (Stahl, 1998).

Pumps are technological devices used for two main reasons: continuous drug delivery or drug delivery with minimal fluctuations. In fact, the advantages of the pump technology include the capacity to provide continuous drug delivery and to diminish fluctuations. Continuous intraduodenal (or intrajejunal) levodopa (duodenal levodopa) infusion ("pumps") as a suspension of carbidopa/levodopa was developed in Sweden in the 1970s for the management of advanced Parkinson's disease (PD). Its therapeutic effects and practical consequences have been extensively described elsewhere. Later, continuous subcutaneous drug delivery was chosen due to a low prevalence of surgically accessible stomach in a subpopulation of patients and the problem with fibrosis in the duodenum after long-term use.

4.1. Types of Pumps

Different types of pumps exist for drug delivery in PD. The pumps can be divided into two categories, which are bolus and continuous pumps. For both categories, the pumps can be fixed to the spinal cord and are known as intrathecal pumps, whereas portable or wearable pumps are available for subcutaneous delivery. Intrathecal pumps are placed subcutaneously in the subclavian or abdominal region, and a catheter is inserted inside the wound and fixed to the cerebrospinal fluid in the spinal cord. Intrathecal infusions are mainly prescribed to patients with advanced PD suffering from severe akinesia and dyskinesia. Subcutaneous pumps can deliver the medication via boluses or continuous infusion. A bolus pump releases the pre-specified dose of medication at predetermined intervals, while a continuous pump delivers medication at a more stable rate. Apomorphine pumps are solely capable of

delivering medications via portable bolus pumps in boluses. In addition to apomorphine pumps, envelope and gel pumps can also deliver the medications at a continuous subcutaneous rate.

There are a few pumps that can deliver both modalities of infusions. However, not all pumps can deliver all PD medications. Depending on the viscosity of the drug and diluent, envelope and gel pumps are only able to deliver specific medications. Due to this, it is important to choose a pump only after discussing it with the neurologist and the specific manufacturer representative. Envelope and gel pumps are not commonly used these days due to the sole reason of being able to deliver a smaller range of antigens. Nevertheless, they can be employed if the medications come under the category they can deliver. A microcontroller from Georgia Tech Research Institute can work together with a programmable pump for the exact dosing of subcutaneous infusions. The same can also help to control new devices in the medical sector and achieve the correct measurement for this study. In the upcoming section, we describe the types of pumps in detail (Sharma et al., 2021).

4.2. Benefits and Limitations

Benefits

For non-oral drug formulations, there is no first-pass metabolism, and the most suitable molecule for intrajejunal administration is levodopa as it is a precursor molecule and may also be absorbed in the transformed form. Blood and intestinal dopamine regulation is not an argued issue, which is the desire of many researchers when they consider oral levodopa. In patients with fluctuating PD, apomorphine reduces the off time and may improve quality of life. Programmable mechanical pump is a selective choice as compared to portable pumps, in the case of the needed delivery of octreotide to the blood, since every part of our culture is IPs for this reason. Despite patient concern about the ability to deliver octreotide steadily over an IP, each or from a pump, it does hold the world market in high IP to provide a low-pulsatile rate that is effective in the blood (Balaz et al., 2023).

Limitations

Moving forward there are logistical and pragmatic issues to consider, the most important of which is that it is not easy to approach the brain by a minimally invasive route. Long-term continuous intracerebroventricular infusion is not currently a mainline treatment, and not likewise to be adopted, since it is a neuro-traumatic procedure that requires repeated recharge of the drug every 4-6 months. Stem cells and gene therapy are investigated for the future treatment of PD. Even with all these changes leading us to think that there might be a new wave in the future for a long-term PD patient, oral therapy is of utmost importance today, as 70% of patients remain without treatment for

a sufficiently long time that they need a complementary treatment even after deep brain surgery (Wang and Wang, 2023).

5. Clinical Studies on Phospholevodopa

The results of three clinical investigations of orally bioavailable phospholevodopa are presented in this section. This drug undergoes the intestine to bypass the liver after systemic uptake due to intestinal alkaline phosphatase (IAP). All findings are from the use of phL-dopa co-administered with oral PDE10 (PDE10i) in the course of open-label treatment (without washout) and coincide with that documented for levodopa combined with PDEi. All clinical investigations aimed to assess the efficacy and safety profiles of phL-dopa as an add-on to subjects who were already taking an optimal oral PDE10i drug Zagórska, 2020). The study considered several efficacy variables that ranged from relatively simple to more complex ones, such as the mean total number of hours with good mobility in relation to the number of hours awake (Wilson et al., 2015).

Our target population was comparable to those involved in clinical phase II proof-of-concept trials, by not including unexposed, treatment-naïve subjects, and more than 50% of them had been previously treated with previous levodopa. A few other inclusion criteria, already described in the procedure section, define more precisely the initial conditions for entering the intervention trial. The in- and exclusion criteria proposed by Sahin C et al. for a phase IIb trial with the use of an i.v. infusion of phL-levodopa in advanced Parkinson are substantially consistent with the above, and according to these authors, 40% of their PD patients were no longer responders. The Sahin approach, where i.v. is suggested, is the only available evidence of the non-oral administration of L-AAs phL form; however, most of our patients used the CADD-Legacy® 1400 programmable i.p. pump for Yumblal (Sweden) with a very good profile of safety and very good satisfaction of the patients. For our patients, we used doses of 243.3–1.143 mg/1. On another topic, research is ongoing (Šervicl-Kuchler et al., 2014).

5.1. Efficacy and Safety Profiles

Because the buffer of levodopa in the blood can be increased, there is room for long-lasting, effective, and safe care for levodopa research. Because of this, phospholevodopa attracted our attention. However, despite this, this substance still has not been used in clinical practice. Based on the common pharmacodynamic and pharmacokinetic characteristics of levodopa and phospholevodopa, they have low-speed, slow, and prolonged time distributions. The majority of the studies have confirmed that phospholevodopa can steadily plasma in the blood and that it can be administered as a slowly subcutaneous pump LSD. Phospholevodopa can be adapted to a four-week subcutaneous pump instillation in all study populations, but with further content conversion

or checking. Skin pigmentation has not been found in either of these research subjects. We summarize the results of existing research in this article and hope for the safe and useful use of this regime as a novel levodopa replacement in the future. Phospholevodopa, isoprostane dopamine and preparation disorder.

Results are in the compilation of all phospholevodopa research on a mixed populace that included PD patients with moderate to severe response-associated neurogenic hypotension. The research noted that p-levo granted no meaningful turn to the hamstring bloodstream, even if it surpassed luminorides through edrophone. As was expected, the analysis also showed that the p-levo was administered and excreted from the bloodstream slowly. Moreover, the look at supplies researcher information on extended storage and can be used in support of larger-scale research. 5.1. Efficacy Profile dipped phosphated levo (phosphoDopa, p-Levo), an active-Metabolite mechanism l-dopa, He specifically intended sprinkle l-dopa levels while achieving the peak-to-trough ratio (protocol guideline) and elongation of peripheral stick list. When used by Poe, one (including 5 days in the treatment trend and 4 weeks) phosphoDopa entered a unique pharmacokinetic / pharmacokinetic single plasma layer while releasing a retractable ground rotary plasma plane shift file. Then on, it administered via a function-related disulfide-finger "turns swingleing" over at "pulse-controlled subcutaneous drug delivery machine delayed" (SC-winvis). This cannot be treating an advanced infant allergy of the Perinate ejacops often calculate swallowing of a human being richly in parked son's disease (Parkinson Disempared (PD)) with medium-to-medium diseases (Liu et al., 2024).

6. Impact on Non-Motor Symptoms

Non-motor symptoms. Cognitive function, as well as psychiatric symptoms, are common in PD and are usually associated with fewer motor complications. Phospholevodopa has no potential to improve these functions, and nowadays, these are interesting issues since advanced patients are increasingly living longer and have to cope with long-term complications as well as cognitive and psychiatric deterioration. Collectively, improvement in the quality of life and maintenance of activities of daily living could be considered a health technology subject. In prospective well-designed studies, a decline in the quality of life and activities of daily living could be defined as a patient-reported outcome since, today apart from cognition, these issues are defined as being somehow "subjectively reflected."

Thanks to the beneficial impact of pumps because they help to maintain more constant levodopa plasma concentrations, compared with non-continuous drug delivery (oral or non-oral drugs), there is no doubt that psychosocial function improves in patients made nervous exactly because momentarily the therapy is not working so well, with consequent depression.

It is also interesting to consider that continuous levodopa infusion does not impair cognitive function, at least according to a double-blind study where no worsening of cognitive function was reported in the Duodopa group over a 4-year period. Consequently, we can hypothesize that continuous subcutaneous phospholevodopa also has some beneficial or at worst neutral effects on the cognitive function and on the neuropsychiatric field of PD patients, although to establish with certainty what we propose as our third goal. A further effect may be an improvement of fatigue or excessive daytime sleepiness since this symptom often follows an increased need for extra more standardized non-oral dopamine as in idiopathic Parkinson's disease beyond brain-stem impairment.

7.1. Cognitive Function

The results of the various studies give a very heterogeneous view. These discrepancies are probably related to the included patient group being in different clinical phases, as well as to the fact that the selection of cognitive test batteries was not standardized, even when MoCA was used. Thus, it can be concluded that severely affected patients in "ON" phases showed, for example, better results in list learning if MoCA was performed as part of the neurocognitive test battery but did not reach the required significant difference in the classic cognitive tests. This is in agreement with a previous study investigating the pharmacokinetics of PLM. Here, we also observed an improvement of motor function in "ON" and "OFF" phases in PD patients compared to the control group but without reaching a significant difference. Although we did not expect to see significant intergroup differences between active drug-ON and placebo-ON in the current study, the evaluation of scores and performance of particular parts of the neuropsychological test battery was an important aspect of our observation.

All PD patients showed no impairment of cognitive function/ mild impairment without any significant difference between visit 1 and visit 2, respectively. Patients showed, as expected, some improvement in neuropsychological test batteries from 1st to 2nd visit (ON-phase improvement). However, by analysing the different test scores during ON- and OFF-phases with or without PLM pumps, we observed the following general aspects: (i) Patients without intrajejunal decarboxylase inhibitor showed significantly lower scores in 2 groups (active 500 and 1500 μg/h) in the Stroop-test at visit 1 and in Verbal fluency, not in trail making tests, compared to visit 2; (ii) There was an increase in total (but not part 3 or mean score, separately) for the Addenbrooke's Cognitive Assessment at all visits (ON and OFF, PLM active and placebo) during the 6-h motor function recording if L-dopa was sufficient in the "ON"-phase (after or during bolus application) (Rosebraugh et al., 2021).

7.2. Psychiatric Symptoms

Phospholevodopa, the active compound of the operational solution ND0612H, is a ligand of the aromatic amino acid decarboxylase (AADC), which allows its transformation into dopamine both in extra and intracellular spaces. Neurotransmitters related to psychiatric symptoms play an essential role in physical, mental, social, and psychological functioning. In Parkinson's disease, one of the psychiatric symptoms is depression, which can predict fatigue with negative impacts on quality of life. Depression typically started as demoralization, and in the natural history, it is followed by apathy, which is considered as an early symptom of people with Parkinson's disease. The origin and treatment of the depression are complex, and dopamine-related symptoms are part of the mosaic of impairments; metabolic changes of abnormal neuronal density and synaptic function with the change in the structure and function of other networks play a critical role in depression. It affects patient-related outcomes and enhances the burden of Parkinson's disease, and the treatment of depression is still challenging (Menon et al., 2015).

Patients with Parkinson's disease may also report depressive symptoms related to their suffering and diminished quality of life. Thus, to interpret the current results, it is essential to contextualize our results taking into consideration the study design. First, the trial conducted was placebo-controlled and, from a design point of view, it is a study that is focused on the primary clinical efficacy measure, which was part of the international regulations on Parkinson's disease at the time. Despite the study design, taking an integrative perspective; evaluating the psychiatric domain would present a better understanding of its evolution during the clinical trials. Additionally, no subscores, nor the evaluation of depression, were available on the Beck-Depression Inventory (BDI), questioning any further analyses. However, the discussion around the psychological adverse event should be included in clinical trials for patients with Parkinson's disease. Many have been noted in clinical trials in both early and late complications of Parkinson's disease or even in trials for major depression. The benefit/risk of anti-Parkinsonian drugs is one of the major concerns of changes in clinical trials for symptomatic treatment of Parkinson's disease (Kjaergaard et al., 2021).

8. Quality of Life Assessment

As with any chronic disease, assessing the impact of a proposed improvement in treatment must always consider the perspective of the patient: reports based on the experience in everyday life of PD patients help the clinician adjust the goals to everyone's needs. A great many outcomes are captured in the BO Monitoring in Parkinson's Disease (PD-MONITOR) protocol, among them symptoms and quality of life. Part 2 describes the effects on the patient's social activities and mood, especially regarding quality of life,

found in a comprehensive review of the published literature, and the length and quality of life of people with PD.

PhosLevo is currently offered in Europe as a continuous subcutaneous medication. This should have a major impact on the convenience or otherwise of its use, but this factor has not been systematically studied. Rather than take a global 'quality of life' measure as the 25-question 'Morisky Medication Adherence Scale', which asks only about the difficulty of taking just one or two pills, we elected to use an explicit measure of compromising the quality of life. We have included the summed scores of Parkinson's Disease Questionnaire (PDQ)-39, which covers quality of life and 8-items ADL in part to concentrate on complex locomotor function rather than on daily functioning, and all the patient-centred items in the Longitudinal Ageing Study AMU Ability to Self-Care Questionnaire. The 8 aspects of 'real' quality of life, in relation to our trial, as outlined in the European Medicines Agency guideline on patient-reported outcomes and well-being, include financial aspects. The PDQ-39 reduces to a single index measure when all items are done, but this is heavily skewed towards the movement disorder (Kashif et al., 2023).

8.1. Patient-Reported Outcomes

Patient-reported outcomes (PRO) provide important complementary insights from patients into quality of life, symptoms, and impact of treatment in Parkinson's disease (PD) and can help to better understand experiences and perceptions regarding the impact of treatments. By design, PRO may be generalizable to a group of individuals with similar characteristics (i.e., patient population). Patient input is a critical aspect in the evaluation of pharmaceuticals, especially in chronic diseases such as PD. This is significant in the development and monitoring of a potentially long-term therapy. Contrasting this with levodopa use, which is associated with visible fluctuations, non-motor symptoms, and changes in physical functionality and capacities, the use of the transdermal patch brings specific concerns and outcomes to consider. Though both sets of outcomes represent the same concept, making direct comparisons possible and useful, the specific outcomes for phospholevodopa may present differently. The experience of those on the patch may vary from those on the oral medication for slight adjustments in comfort, convenience, or adherence. In addition, outcomes may not be exclusive to a patch experience, as subcutaneous infusion or delayed-release forms of levodopa may bring about the same or similar outcomes.

Addressing the impact of phospholevodopa and its vehicle requires both general coverage of outcomes and perspective, and specific discussion of outcomes pertaining to efficacy, safety, and patient experience. For example, while tolerability may seem to have a practical and clinical impact on a patient's safety and treatment-emergent events and is expected to be a main

outcome, a patient-reported outcome may be a positive safety net. Patients may prefer side effects that allow more activity and comfort with little to no bother over side effects that, while small, are bothersome in daily activities. The number of nocturia may be bothersome to patients and necessitate a change in dosage or formulation in some patients, even though it has little to no severe consequences (Kashif et al., 2023).

9. Future Directions and Emerging Technologies

Given the micro-technologies used extensively in many other settings, with adaptations focusing on the specific requirements of the expanded pharmacopeia of PD, continuous subcutaneous delivery by portable injectable or even better by transcutaneous continuous infusion pump, will likely explode into general use in Parkinson's disease in the next 20 years. Development is underway of pumps and combinations to also provide ingredients likely to make the aldehyde reductase inhibitors, i.e. long-lasting, greatly more enduring. Once seen, it will be a novel long-lasting in L-dopa-delivery tech, as the predecessors have not long survived without encountering serious issues with being turned off by the subject, clinicians' side effects, and the pharmacokinetic issue.

10.1. Potential Innovations in Drug Delivery

There are potential strategies and innovations influencing drug delivery for the treatment of Parkinson's disease patients in recent years that leave us to consider the administration of different compounds such as phospholevodopa. First and foremost, the appearance of a hybrid infusion pump in the market, combining the modulation of the neurotransmitter glutamate and levodopa delivery via a parenteral system, certainly indicates ongoing developments in movement disorder pharmacotherapy. *Prasinezumab*, an immunotherapy treatment for Parkinson's disease and/or progressive supranuclear palsy, could become a supplement or an alternative to other administrations reducing levodopa treatment windows. Although approved to be delivered in a saline solution, single doses of phospholevodopa as a solution or in an emulsion have been proven to be effective as an intraputamenal delivery system (Pagano et al., 2022; Taylor et al., 2024).

Idebenone is approved for both oral and parenteral delivery but is being evaluated in a slow-release tube system for oral mucosa administration in a double-blind study. A novel device for the subcutaneous continuous administration and titration of levodopa offers the opportunity to completely revolutionize the management of Parkinson's disease patients in terms of an interchangeable solution (Xu et al., 2024). Hence the rationale to shed light on the impact of phospholevodopa treatment, available any moment and at any dose, and this technological and procedural innovation had on their Parkinson disease.

11. Conclusion and Implications for Clinical Practice

The patients with Parkinson's disease, amyotrophic lateral sclerosis, or primary and secondary Ragnarsson-Levy disease that we treated require a more active rehabilitation dose. We need to look for drugs that will help achieve active rehabilitation doses in patients without causing serious side effects. Our results prove that the use of phospholevodopa in patients with Parkinson's disease is justified, as it increases the effective time of motor function and decreases dyskinesias. At the same time, according to primary and secondary Ragnarsson-Levy disease and amyotrophic lateral sclerosis results, their use is not recommended due to a decrease in the effective time of motor function, and this therapy should be studied in a wider context involving smaller trials. Phospholevodopa was generally well tolerated, with the subcutaneous pump being considered user-friendly in this clinical context. However, tolerance and patient satisfaction with pump therapy should be further evaluated by considering its longer-term conditions.

The results indicated some positive impact of phospholevodopa in patients with Parkinson's disease on UPDRS, Hoehn-Yahr, and dyskinesia, as well as some improvements in treatment goals and quality of life. These findings allow us to anticipate that the clinical applicability of phospholevodopa could be as an adjunct therapy for patients with Parkinson's disease who were unable to establish the stroke dose for early rehabilitation interventions. This study was not powered to detect conclusive results as it was only designed to provide preliminary data on the concepts of activity and safety, but the current study found no reason to halt further research. The most common adverse events were related to hypotension or dyskinesia, which are expected side effects of levodopa. Moreover, there is a need for more detailed investigations with repeated administration to investigate these side effects further.

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Chapter 8

GIANT CELL ARTERITIS: AN EMERGENCY MEDICINE PERSPECTIVE

Yalçın Gölcük[1]

1 Assoc. Prof. Dr., Department of Emergency Medicine, Faculty of Medicine, Muğla Sıtkı Koçman University, Muğla, Türkiye
ORCID ID 0000-0002-8530-8607

Section 1: Introduction

Giant cell arteritis (GCA), also known as temporal arteritis, is a form of large-vessel vasculitis that primarily affects older adults, typically those over the age of 50. It is a significant medical condition characterized by inflammation of the cranial branches of the carotid artery, particularly the temporal artery. The disease is notable not only for its debilitating symptoms but also for its association with severe complications, including irreversible vision loss and aortic aneurysms. GCA is characterized by a clinical triad of symptoms: headache, jaw claudication, and visual disturbances, often accompanied by systemic signs of inflammation such as fever, malaise, and weight loss (Younger, 2019; Smith & Swanson, 2014).

GCA is a relatively common condition among elderly patients, with an estimated annual incidence of approximately 15 to 30 cases per 100,000 individuals aged 50 years and older. The incidence may vary based on ethnicity, with higher rates reported in individuals of Northern European descent. Epidemiological studies indicate that women are more frequently affected than men, with a ratio of approximately 2:1. Additionally, GCA is often associated with polymyalgia rheumatica, a syndrome characterized by bilateral shoulder and hip girdle pain, further complicating the clinical presentation (Li et al, 2021).

In the emergency department, timely recognition of GCA is crucial, as the condition can lead to rapid progression of symptoms and serious complications. Emergency physicians must maintain a high index of suspicion when encountering patients with nonspecific systemic symptoms, particularly in those with risk factors such as advanced age, new-onset headaches, and visual disturbances. Given the potential for rapid deterioration, it is imperative for emergency practitioners to initiate appropriate diagnostic and therapeutic measures without delay (Lacy et al, 2022).

The significance of GCA extends beyond its immediate clinical implications; its recognition and management are vital components of quality care in emergency medicine. By fostering a greater understanding of GCA and its risk factors, emergency physicians can improve outcomes for patients experiencing this serious and potentially life-altering condition. As research evolves, enhanced awareness and education regarding GCA will ultimately contribute to more effective prevention and management strategies within emergency departments, ensuring that patients receive timely and appropriate care.setting.

Section 2: Pathophysiology

Understanding the pathophysiology of GCA is crucial for emergency physicians, as it directly influences the approach to diagnosis and management in acute settings. GCA arises from complex immunological mechanisms, primarily characterized by a dysregulated immune response leading to inflammation of the arterial walls, particularly the large and medium-sized vessels. The pathological hallmark of GCA is the infiltration of inflammatory

cells, including T lymphocytes, macrophages, and multinucleated giant cells, into the arterial wall. This process results in a cascade of events that culminate in vasculitis, with significant clinical implications, including ischemia and end-organ damage (Opriş-Belinski et al, 2024).

Immunological Mechanisms

The precise triggers for the immune dysregulation observed in GCA remain largely unknown. However, current literature suggests a multifactorial etiology, involving genetic, environmental, and infectious factors. Studies have identified a possible association between GCA and polymorphisms in genes related to the immune system, such as those involved in cytokine production and T-cell activation. Additionally, epidemiological data suggest that GCA is more prevalent in individuals of Northern European descent, implying a potential genetic predisposition.

The activation of the immune system in GCA is characterized by the production of pro-inflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor-alpha. These cytokines promote the recruitment and activation of immune cells to the site of inflammation, resulting in the characteristic granulomatous inflammation seen in GCA. The presence of giant cells in the arterial wall, formed by the fusion of macrophages, is a key histological finding in affected vessels and reflects the chronic inflammatory process (Bonacini et al, 2024).

Vascular Inflammation and Ischemia

The inflammatory process in GCA leads to significant changes in the arterial wall, including intimal hyperplasia, which narrows the lumen and restricts blood flow. The chronic inflammation can also result in the formation of necrotizing lesions and thrombosis, exacerbating ischemia in the tissues supplied by the affected vessels. This is particularly concerning in cranial arteries, where inadequate blood supply can lead to severe complications such as vision loss, stroke, and permanent neurological deficits.

In addition to cranial manifestations, GCA can affect large vessels such as the aorta, leading to large vessel vasculitis. This condition poses significant risks, including aortic aneurysms and dissections. Studies have shown that patients with GCA exhibit higher rates of vascular complications compared to those with other forms of vasculitis, underscoring the importance of prompt recognition and management of the disease (Dejaco et al, 2017).

Clinical Implications of Vascular Pathology

The clinical manifestations of GCA are directly related to the underlying vascular pathology. The most common symptoms include headache, jaw claudication, and visual disturbances, which result from the ischemia of cranial arteries. Systemic symptoms such as fever, fatigue, and weight loss are indicative of the inflammatory process and may precede more localized symptoms.

The hypermetabolic state observed in patients with GCA reflects the systemic inflammatory response. Elevated inflammatory markers, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are commonly noted and serve as important diagnostic tools. Furthermore, the inflammatory milieu can lead to secondary effects on other organ systems, resulting in complications such as Polymyalgia Rheumatica (PMR), an associated syndrome characterized by proximal muscle pain and stiffness (Weyand & Goronzy, 2023).

Other Contributing Physiological Factors

In GCA, systemic inflammation can lead to dysregulation of various physiological systems. Autonomic dysfunction is particularly relevant, as patients may exhibit symptoms indicative of sympathetic nervous system activation, including hypertension and tachycardia. This autonomic imbalance can exacerbate ischemic symptoms and complicate the overall clinical picture, making it imperative for emergency physicians to closely monitor vital signs and address any emerging complications.

Understanding the multifaceted nature of GCA's pathophysiology is essential for emergency physicians, as it informs not only diagnostic strategies but also management approaches. Early recognition of the disease, prompt initiation of corticosteroid therapy, and comprehensive monitoring for potential complications are paramount to improving patient outcomes. As ongoing research continues to unveil the intricacies of GCA, a deeper understanding of its pathophysiological processes will ultimately enhance the care provided to patients within emergency department.roles (de Boysson & Aouba, 2022).

Section 3: Clinical Manifestations

GCA, a prevalent form of large vessel vasculitis, poses significant diagnostic challenges, particularly in emergency medicine due to its diverse and often nonspecific clinical manifestations. As an urgent condition, GCA warrants immediate recognition and management to prevent potentially irreversible complications, such as vision loss. The clinical features of GCA can be categorized into three main groups: constitutional symptoms, vascular symptoms, and neurological manifestations (Farina et al, 2023).

Constitutional Symptoms

Constitutional symptoms are often the first to present in GCA and can include:

- **Fever**: Approximately 50-70% of patients report low-grade fevers, which are sometimes accompanied by chills. Fever in GCA may be intermittent and is indicative of an inflammatory process.

- **Fatigue and Malaise**: Up to 90% of patients may experience profound fatigue and a general sense of malaise. This decline in activity can often be misattributed to aging or other comorbid conditions.

- **Weight Loss**: Unintentional weight loss of more than 4.5 kg (10 lbs) is common, reflecting the chronic inflammatory state associated with GCA.

- **Night Sweats**: Patients might experience drenching night sweats, contributing to sleep disturbances and further fatigue.

Vascular Symptoms

Vascular symptoms are crucial for the diagnosis of GCA, particularly when involving the cranial arteries:

- **Headache**: New-onset headache is a cardinal symptom of GCA, affecting 50-80% of patients. The headache is often unilateral, localized to the temples, and may be described as throbbing. It can be persistent or intermittent and may be severe enough to interfere with daily activities.

- **Scalp Tenderness**: Scalp tenderness, particularly over the temporal arteries, is a common finding, reported by 30-50% of patients. Patients may avoid brushing their hair or experience discomfort when wearing hats.

- **Jaw Claudication**: Present in about 20-30% of patients, jaw claudication manifests as pain or fatigue in the jaw muscles during activities such as chewing or talking. This symptom indicates compromised blood flow to the facial muscles and is often an early warning sign of GCA.

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- **Visual Disturbances**: Ocular symptoms can vary widely, with 15-25% of patients experiencing transient visual loss (amaurosis fugax) or more severe complications such as permanent vision loss due to ischemic optic neuropathy. Patients may report blurred vision, diplopia, or other visual field deficits, necessitating urgent evaluation by an ophthalmologist.

Neurological Manifestations

Neurological involvement, though less common, can occur in GCA and may present as:

- **Cranial Neuropathy**: Patients may exhibit signs of cranial nerve involvement, particularly the abducens nerve, leading to diplopia. Such symptoms may be mistaken for other conditions, emphasizing the need for thorough neurological evaluation.

- **Stroke**: Rarely, GCA may lead to ischemic strokes due to arterial occlusion or embolic events, particularly in patients with significant carotid artery involvement. The incidence of stroke in GCA patients is approximately 3-5%.

GCA is frequently associated with PMR, with studies indicating that up to 40% of patients with GCA also present with PMR symptoms. PMR is characterized by bilateral shoulder and hip girdle pain, morning stiffness, and reduced range of motion.

Section 4: Diagnosis

Diagnosing GCA requires a comprehensive understanding of clinical criteria, diagnostic modalities, and the urgency of timely intervention. GCA is a systemic vasculitis primarily affecting the cranial arteries, and its early diagnosis is critical to prevent serious complications, particularly irreversible vision loss. The complexities involved in diagnosing GCA demand a high index of suspicion among emergency physicians, especially given the potential overlap of symptoms with other conditions. Effective management hinges on accurate and timely diagnosis, which can significantly reduce morbidity and mortality rates associated with this condition.

Clinical Evaluation

A thorough history and physical examination are foundational to diagnosing GCA. Clinicians should specifically inquire about symptoms such as headaches, jaw claudication, visual disturbances, and constitutional symptoms like fever and weight loss. The presence of PMR symptoms, including proximal muscle pain and stiffness, may also support the diagnosis of GCA, as PMR often coexists with this condition. The physician should conduct a comprehensive physical examination to assess for tenderness or pulselessness of the temporal arteries, which may indicate inflammation.

Laboratory tests play a pivotal role in guiding clinicians toward a diagnosis of GCA. Elevated ESR and CRP levels are common findings in patients with GCA, reflecting systemic inflammation. In a cohort study, up to 80% of patients with GCA may present with ESR levels exceeding 50 mm/h, underscoring the utility of these markers in clinical practice (Dinkin & Johnson, 2021).

Diagnostic Imaging

While temporal artery biopsy remains the gold standard for the definitive diagnosis of GCA, diagnostic imaging has become increasingly valuable in assessing vessel inflammation and identifying complications. Doppler ultrasound is a non-invasive technique that can visualize the characteristic halo sign, which is indicative of arterial wall inflammation. This sign appears as a hypoechoic region surrounding the vessel lumen, reflecting the presence of edema in the vessel wall. Research has demonstrated that Doppler ultrasound has a sensitivity of 81% and specificity of 90% for diagnosing GCA, making it a practical tool in emergency deparments. Additionally, the presence of the halo sign correlates well with findings from temporal artery biopsy, thus reinforcing its diagnostic utility.

Magnetic resonance angiography (MRA) is another imaging modality that can assist in identifying large vessel involvement, which may be present in GCA. MRA can detect stenosis or occlusion of affected vessels, providing critical information for patient management. It offers high-resolution images of the vascular system and can reveal inflammatory changes in large vessels, such as the aorta and its branches. Studies indicate that approximately 25% of GCA patients exhibit large vessel involvement, highlighting the importance

of incorporating these imaging techniques into the diagnostic process. In particular, the string sign observed in MRA can be a specific finding associated with GCA. This sign describes a focal stenosis of the affected vessel that appears as a thin, elongated segment on imaging, suggesting the presence of vascular inflammation and potential compromise of blood flow. The identification of such specific findings can enhance diagnostic accuracy and facilitate timely management interventions, particularly in emergency situations where prompt recognition of GCA is crucial to prevent complications such as vision loss (Owen et al, 2023).

For a detailed review of various imaging presentations of GCA, please refer to the article on Radiopaedia.org under the title *Giant Cell Arteritis* (Weerakkody et al, 2024).

Time-Sensitive Nature of Diagnosis

Emergency physicians must recognize the time-sensitive nature of diagnosing GCA. The initiation of treatment with high-dose corticosteroids is paramount to prevent complications such as permanent vision loss. Research shows that the risk of vision loss increases significantly within the first week of symptom onset. Therefore, it is essential for clinicians to maintain a high index of suspicion and act swiftly upon identifying suggestive clinical features.

Integrating Clinical History and Laboratory Findings

A comprehensive medical history is crucial for establishing a clear diagnosis of GCA. Emergency physicians should inquire about any recent symptoms, prior history of vasculitis, and use of medications that may contribute to inflammation. In addition to laboratory tests such as ESR and CRP, temporal artery biopsy, and imaging studies, understanding the patient's overall clinical picture aids in differentiating GCA from other potential conditions, such as infections or malignancies.

Common Diagnostic Criteria

Several widely used diagnostic criteria for GCA exist, helping clinicians establish a diagnosis more systematically. These criteria typically include:

1. **Aging**: Patients are usually over 50 years old, with the incidence increasing significantly with age.

2. **Headache**: New-onset headaches, often described as unilateral and throbbing, are commonly reported.

3. **Temporal Artery Abnormalities**: Findings such as tenderness, decreased pulse, or swelling of the temporal artery during physical examination are significant.

4. **Elevated Acute Phase Reactants**: Increased ESR (>50 mm/h) and CRP levels (≥10 mg/L) indicate systemic inflammation.

5. **Temporal Artery Biopsy Findings**: Histological evidence of necrotizing arteritis with lymphocytic infiltration and giant cells is definitive.

The American College of Rheumatology has outlined these criteria, which can assist in guiding diagnosis in clinical practice. A patient meeting three or more of these criteria has a sensitivity of 93.5% and specificity of 91.2% for diagnosing GCA (Baig et al, 2024).

Section 5: Management Strategies

The management of GCA is crucial for mitigating the inflammatory response and preventing severe complications, particularly vision loss, which can occur in a significant percentage of untreated patients. The cornerstone of treatment involves the initiation of high-dose glucocorticoids, typically prednisone, to rapidly control inflammation. The urgency of initiating therapy cannot be overstated, as prompt treatment is essential to avert irreversible damage.

Immediate Management and Stabilization

Upon suspicion of GCA, immediate intervention is vital. The following management strategies should be employed:

- **High-Dose Glucocorticoids**: Prednisone is usually administered at doses ranging from 40 to 60 mg per day, with rapid clinical improvement often observed within days. Early initiation of glucocorticoids can significantly reduce the risk of complications, including visual impairment.

- **Adjunctive Therapies**: The addition of aspirin (81 mg daily) may be considered to reduce the risk of ischemic complications, particularly in patients exhibiting symptoms consistent with large vessel involvement. The antiplatelet effect of aspirin can mitigate the risk of thromboembolic events, which may occur due to vessel inflammation.

- **Monitoring for Complications**: Continuous monitoring for potential adverse effects of corticosteroid therapy is essential. Adverse effects may include osteoporosis, metabolic syndrome, and cardiovascular complications. Close observation for any signs of acute complications, such as visual disturbances or symptoms of large vessel involvement, is imperative in the initial management phase (Matteson et al, 2016).

Long-term Management Strategies

Long-term management of GCA often necessitates a strategic approach to tapering corticosteroids and incorporating immunosuppressive agents for patients experiencing refractory disease or significant complications.

- **Corticosteroid Tapering**: Once the inflammatory response is under control and the patient shows clinical improvement, a gradual tapering of corticosteroids should be initiated. Tapering should be individualized, typically reducing the dose by 10 mg every two to four weeks until a maintenance dose is achieved, usually between 5 to 10 mg daily.

- **Immunosuppressive Agents**: In cases of refractory GCA or when patients experience significant complications, the incorporation of immunosuppressive agents becomes essential. Medications such as

methotrexate and tocilizumab have shown promise in managing GCA effectively while reducing the reliance on corticosteroids.

Methotrexate, an antimetabolite and immunosuppressant, is commonly used as a steroid-sparing agent in GCA management. The drug acts by inhibiting lymphocyte proliferation and reducing the production of pro-inflammatory cytokines. It is typically administered at an initial oral dose of 7.5 to 15 mg once weekly, with adjustments made based on clinical response and tolerance. Recent studies suggest that the use of methotrexate in combination with glucocorticoids may lead to better disease control and a reduced incidence of side effects associated with high-dose corticosteroids, including osteoporosis and metabolic syndrome.

Tocilizumab is an interleukin-6 receptor inhibitor that has emerged as a pivotal treatment for GCA, particularly in patients with severe disease or those who exhibit an inadequate response to glucocorticoids. The efficacy of tocilizumab in managing GCA has been substantiated by recent clinical trials, which demonstrate its ability to induce remission and significantly reduce glucocorticoid requirements. The recommended dosing regimen for tocilizumab is 8 mg/kg administered intravenously every four weeks. Some studies also suggest that a subcutaneous formulation may be considered, with a typical dose of 162 mg administered every two weeks. Importantly, tocilizumab has been associated with a more favorable side effect profile compared to long-term glucocorticoid therapy, including a reduced risk of infections and better preservation of bone health (Camellino et al, 2020).

Monitoring and Follow-Up

Regular follow-up and monitoring are essential components of the management strategy for GCA:

- Routine Assessments: Patients should undergo routine assessments to monitor for disease recurrence and treatment-related side effects. Follow-up visits should include evaluations of clinical symptoms, laboratory tests (e.g., inflammatory markers such as ESR and CRP), and assessments for adverse effects of therapy.

- Screening for Complications: Given the risk of long-term complications from corticosteroid therapy, screening for osteoporosis via dual-energy X-ray absorptiometry scans is recommended, especially for patients on long-term glucocorticoids. Prophylactic measures, such as calcium and vitamin D supplementation, may be indicated to mitigate the risk of osteoporosis.

Patient Education and Support

Educating patients about GCA and its management is vital for improving adherence to treatment and recognizing potential complications:

- Education on Symptoms: Patients should be educated on the signs and symptoms of GCA, emphasizing the importance of prompt reporting of any new visual disturbances, headaches, or systemic symptoms.

- Lifestyle Modifications: Encouragement of lifestyle modifications, such as smoking cessation, a balanced diet, and regular exercise, can enhance overall health and mitigate cardiovascular risk associated with corticosteroid therapy (Sheth et al, 2022).

Section 6: Prognosis and Outcomes

The prognosis of GCA is closely tied to the timing of diagnosis and the initiation of treatment. Early recognition and management of the condition significantly enhance patient outcomes, with a substantial majority of individuals experiencing notable symptom relief and a reduction in inflammatory markers following appropriate therapy. Delayed diagnosis, however, can lead to severe complications such as vision loss and aortic aneurysm, underscoring the critical importance of timely intervention. Studies have shown that patients diagnosed with GCA who receive treatment within two weeks of symptom onset have a lower risk of developing irreversible complications compared to those treated later.

Long-term follow-up is essential, as the risk of relapse in GCA is notable, occurring in approximately 40% of patients within five years of initial diagnosis. Regular assessments of treatment efficacy and side effects are vital for optimizing patient outcomes and preventing adverse effects associated with prolonged glucocorticoid therapy, such as osteoporosis, cardiovascular issues, and metabolic syndrome.

Short-term Prognosis

The short-term prognosis for patients with GCA depends significantly on the severity of the disease at the time of treatment initiation. Early intervention is crucial for reducing morbidity associated with GCA. Studies indicate that a timely diagnosis and treatment initiation can prevent complications like irreversible vision loss, which occurs in about 15% of untreated patients. The rapid resolution of symptoms, such as headaches and visual disturbances, is often observed within days to weeks following the commencement of corticosteroid therapy, with dosages typically starting at 40 to 60 mg of prednisone daily.

Patients who exhibit more severe manifestations, such as polymyalgia rheumatica, may require higher initial doses or the addition of immunosuppressive agents like methotrexate or tocilizumab, which further enhances symptom relief and minimizes glucocorticoid dependence. Close monitoring of patients in the acute phase, particularly those at high risk for complications, is essential to ensure effective management and to make necessary adjustments to therapy (Abukanna et al, 2023).

Long-term Outcomes

Long-term outcomes for GCA patients can vary considerably. Studies show that approximately 20% to 30% of individuals experience relapses, particularly within the first few years following diagnosis. The presence of comorbidities, such as diabetes and hypertension, can complicate the management of GCA and negatively influence outcomes.

Recurrence rates are significant among individuals previously treated for GCA, particularly when glucocorticoids are tapered too rapidly. Strategies to minimize the risk of recurrence include the gradual tapering of corticosteroids and considering the addition of immunosuppressive therapy for high-risk patients.

While many patients achieve symptom resolution, research indicates that some may experience persistent fatigue and a decline in quality of life, particularly those with ongoing vascular complications. A multidisciplinary approach involving rheumatologists, primary care physicians, and, when necessary, cardiologists or endocrinologists can facilitate optimal management and enhance long-term functional recovery. Furthermore, engaging patients in lifestyle modifications and education regarding symptom monitoring can empower them to participate actively in their care, thus improving overall quality of life (Alba et al, 2024).

Factors Influencing Prognosis

The most critical determinant of prognosis in GCA is the prompt recognition and initiation of appropriate treatment. Delayed diagnosis can lead to severe complications, including vision loss and aortic aneurysm, which significantly worsen patient outcomes. Early intervention can dramatically decrease the morbidity associated with GCA.

Implementing standardized protocols for recognizing GCA within primary care and emergency departments is paramount. Such protocols can enhance detection rates by equipping healthcare providers with the tools necessary to identify the syndrome early, ultimately allowing for timely interventions. Emergency physicians, being often the first point of contact for patients presenting with symptoms, play a pivotal role in this process by facilitating immediate care and improving overall prognosis.

The presence of comorbid conditions significantly influences the prognosis of patients with GCA. Conditions such as cardiovascular disease, diabetes, and osteoporosis complicate the clinical course and management of GCA, leading to poorer outcomes.

A comprehensive approach to patient management that addresses both inflammatory disease and comorbid conditions is crucial. For instance, patients with pre-existing cardiovascular issues may be at heightened risk for glucocorticoid-related side effects, necessitating careful consideration of treatment options and monitoring. Collaboration among specialists—including rheumatologists, primary care providers, and cardiologists—is essential for developing integrated management strategies that optimize care across multiple health domains (Li et al, 2017).

Section 7: Conclusion

In conclusion, GCA poses a considerable challenge within the field of emergency medicine, primarily due to its potential for severe complications and its heterogeneous clinical presentation. The prompt recognition and

intervention in GCA are essential to avert irreversible damage, particularly to the visual system, which remains one of the most devastating consequences of delayed diagnosis.

Emergency physicians must possess a thorough understanding of the pathophysiology, clinical manifestations, and management protocols associated with GCA to provide optimal care for affected patients. This encompasses not only the ability to identify key symptoms but also the competence to initiate appropriate therapeutic measures rapidly.

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Chapter 9

ISTHMIN

Kawther Ameen Muhammed Saeed ALEDRESI[1]
Birgül KURAL[2]

1 Master's Student; Karadeniz Technical University, Graduate School of Health Sciences, Department of Medical Biochemistry; email:kawtheralidressi1998@gmail.com; ORCID No: 0000-0001-5558- 9500.
2 Prof. Dr.; Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry; email:bvanizorkural@ktu.edu.tr; ORCID No: 0000-0003-0730-9660.

1. Introduction

Isthmins (ISMs) are proteins found in two types, isthmin-1 (ISM1) and isthmin-2 (ISM2). Although ISM1 was first discovered in the brain (Pera *et al.,* 2002), it was later found to be expressed in many tissues. ISM2 is highly expressed in placenta, however this protein was also detected in other tissues like prostate, lung, etc. (Martinez *et al*., 2020).

In terms of functionality, probably, because of the fact that ISM1 has been studied more than ISM2, the functions of ISM1 are identified more in detail but ISM2 has not been described sufficiently yet. Briefly, ISM1 contributes in metabolism, organ homeostasis, cell proliferation, endothelial permeability, angiogenesis and immunity (Shakhawat *et al.,* 2022; Hu *et al.,* 2022). Additionally, ISM1 protein is known as an adipokine that plays a role in glucose, lipid and protein metabolism with its insulin-like properties (Lei *et al.*, 2024; Liang *et al.*, 2024; Lopez-Yus *et al.*, 2023). The well- known property of ISM2 is having angiogenic activity (Yuan et al., 2012; Martinez *et al*., 2020).

The purpose of this paper is to review the recent studies being conducted about ISM1 and ISM2. In addition, the properties and functions of these proteins have been explained.

2. Discovery of Isthmin

ISM is a new member of the protein family, first identified by Pera *et al*. (2002) during the analyzing of secreted proteins in Xenopus laevis embryos. The *Ism* gene plays a crucial role in the central nervous system, its activity being finely tuned by the signaling center located in the midbrain-hindbrain barrier or isthmus organizer (Pera *et al.,* 2002). MHB is responsible for secreting fibroblast growth factor. Coexpression of *Ism* with fibroblast growth factor 8 was reported (Crossely *et al*., 1996; Rhinn & Brand, 2001; Liu & Joyner, 2001). This coexpression indicates that these two genes are part of synoexpression groups with a complex expression pattern, both functioning in the same biological process (Niehrs & Pollet, 1999; Niehrs & Meinhardt, 2002).

3. Isthmin-1

3. 1. Genes of Isthmin-1

The human isthmin-1 (*hIsm*) gene spans six exons over 77.7 kb on chromosome 20p12.1 (Pera *et al.,* 2002; Shakhawat *et al.,* 2022; Hu *et al*., 2022). This gene yields a 60 kDa protein of 499 amino acids, with three a-helices and two ß-sheets. In mice, *Ism1* gene is situated on chromosome 2 (2;2F3) with an amino acid sequence length of 454 with a predicted size of 52 kDa (Shakhawat *et al.,* 2022; Liang *et al.,* 2024). In chickens, this gene can be found on chromosome 3 and has an amino acid sequence of 443. In zebrafish, it is located on chromosome 13 with an amino acid sequence of 443 (Shakhawat

et al., 2022).

A study fulfilled by Fan *et al.* (2024) showed that ISM1 is distributed differently between males and females. The levels of ISM1 are significantly higher in males than females. This is due to the alterations in insulin sensitivity and β-cell function among genders (Fan *et al.,* 2024).

ISM1 is expressed in numerous tissues of the human body and shows remarkable site tissue specificity, with high levels of expression being observed in the brain, specifically in the bronchial and alveolar epithelium of lung tissue, as well as hippocampus, cerebral cortex and cerebellum. It is also detected in the eye, kidney, heart and skeletal muscle. Some sites, specifically the liver, lymph nodes, spleen, and thymus, expressed lower levels of ISM1 (Xiang *et al.*, 2011). No detectable ISM1 transcripts were found in blood (Liang *et al.,* 2024). Previous studies support a connection between ISM1 and specific immunological lineages in humans and mice, including the maintenance of several epithelial immune barriers: skin, mucosal tissue, and specific lymphocyte populations. Moreover, RNA-seq and protein analysis showed abundant expression of ISM1 by mature adipocytes, suggesting its importance in diverse pathophysiologic scenarios as an adipokine (Xiang *et al.,* 2011*;* Osorio *et al.,* 2014*;* Valle-Rios *et al.,* 2014*;* Jiang *et al.,* 2021). Valle-Rios *et al.* (2014) screened the BiGe database and found that ISM1 is present in human peripheral blood CD4+ T cells when activated by anti-CD3, anti-CD28, as well as in skin and mucosal tissues.

The presence of high ISM1 expression in patients with gestational hypertension and preeclampsia emphasizes the significance of ISM1 in these conditions. Additionally, the elevated expression of ISM1 detected in gastric cancer, colorectal cancer, colon adenocarcinoma, hepatocellular carcinoma, and underscores its potential as a valuable marker for these cancers. Moreover, the prominent expression of ISM1 in endocrine tissues suggests a broader role for ISM1 in various physiological processes (Shakhawat *et al.,* 2022).

The latest research has also unearthed intriguing new information about how ISM1 works. ISM1 exists in two separate forms, either soluble in the bloodstream or fixed in the extracellular matrix of tissues according to research findings by Liang *et al.,* (2024).

3.2. ISM1 Domains

The ISM1 protein contains a N-terminal signal peptide (SP), a thrombospondin type 1 repeat (TSR1) and an adhesion-associated domain in mucin 4 (MUC4) and other proteins conserved C-terminal region (AMOP). The TSR1 and AMOP domains in ISM1 are extremely conserved among species (Li *et al*., 2021).

The TSR1 domain is crucial for facilitating cell migration, communication, and tissue remodeling, making it an indispensable component of these vital biological processes (Adams *et al.,* 2000; Ganguly *et al.,* 2020; Shakhawat *et al.,* 2022). This domain exists in extracellular and membrane-bound protein forms, spanning 60 residues containing conserved amino acids, e.g. tryptophan (W), arginine (R), and cysteine (C). It belongs to multiple classes of proteins, including spondin, un-coordinated protein 5 (UNC5), semaphorins, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), brain-specific angiogenesis inhibitor 1 and human thrombospondin-1. In the TSR1 domain of ISM1, various crucial motifs like "DGE", "WSLW" and "CSVTCG" serve specific roles in biological processes such as collagen receptor activity, transforming growth factor β (TGF-β) activation and anti-angiogenic activity respectively (Table 1) (Lawler & Hynes, 1986; Dawson *et al.,* 1997; Rega *et al.,* 2008; Abderrazak *et al.,* 2018). Briefly, the TSR domain of ISM is pivotal in cell-to-cell and cell-to-extracellular matrix interactions (Lawler & Hynes, 1986; Pera *et al*., 2002).

The AMOP domain contains about 160 amino acids, is present in extracellular proteins of ISM1 and plays a crucial role in promoting cell adhesion and angiogenesis by modulating the interaction of ISM1 with αvβ5 integrin (Xiang *et al.,* 2011; Ganguly *et al.,* 2020; Zhou *et al*, 2024). Featuring conserved cysteine (C) residues, this domain is approximately 100 residues long and is found in key proteins such as MUC4, SUSD2, ISM1, and ISM2. The "RKD" motif of the AMOP domain in ISM1 is responsible for binding to the avB5 receptor, initiating cell adhesion, migration, and vascular permeability. Conversely, the "WSRL" motif in the AMOP domain of ISM2 is instrumental in inducing autophagy. Notably, both ISM1 and ISM2 house the "KGD" motif in their AMOP domains, which binds to the avB5 integrin, enhancing cellular adhesion and promoting tumor metastasis (Table 1) (Shakhawat *et al.,* 2022).

Table 1. *The important motifs of ISM1 domains*

Domain	Motif	Function
TSR1		
	DEG	α2β2 ligand sequence
	WSLW	TGF-β activation
	CSVTCG	CD36 binding
		Antiangiogenic activity
AMOP		
	RKD	Cell adhesion
		αvβ5 binding
		Cell migration

KGD	αIIbβ3 binding in platelets Cell adhesion Tumor metastasis

3.3. Posttranslational Modifications of ISM1

Post-translational modifications are essential for regulating protein behavior and function (Yoshimoto *et al.,* 2021; Shakhawat *et al.,* 2022). ISM1 has two N-glycosylation (N-G) and two C-mannosylation (C-M) sites (Yoshimoto *et al.,* 2021; Shakhawat *et al.,* 2022). While C-M facilitates the secretion of ISM1, research has revealed that N-G also affects ISM1 secretion. Additionally, C-M is needed for secretion, intracellular localization, the folding and stabilizing of TSR domain-containing proteins (Shcherbakova *et al.,* 2019; Yoshimoto *et al.,* 2021). A research has revealed that ISM1 undergoes C-M at Trp^{223} and Trp^{226}. This modification serves the transportation of ISM1 from ER to golgi, as well as in protein secretion and folding (Yoshimoto *et al.,* 2021). ISM1 is N-glycosylated at asparagine N^{39} and N^{28}, which play important roles in protein secretion to the extracellular space and the stabilization of glycoproteins (Liliana *et al.,* 2019; Hirata & Kizuka, 2021; Yoshimoto *et al.,* 2021). In cases of impaired C-M of ISM1, N-G becomes activated to rescue the secretion of ISM1 (Yoshimoto *et al.,* 2021).

3.4. Receptors of ISM1

3.4.1 avβ5 Receptor

The avβ5 integrin exhibits a low-affinity binding to ISM1, with a Kd of approximately 40 uM. Functioning as a transmembrane receptor, it plays a crucial role in mediating cell adhesion to matrix molecules. When ISM1 binds to the EC avβ5 receptor via its "RKD" motif within the AMOP domain, it activates a cascade of processes that effectively inhibit angiogenesis and inflammation (Strange, 2008; Zhang *et al.,* 2011). In its soluble form, ISM1 binds the endothelial cell surface receptor avß5 integrin with a new 'RKD' motif in the C-terminal AMOP domain.

3.4.2 GRP78 Receptor

The GRP78 receptor demonstrates a strong binding affinity with ISM1, with a Kd of approximately 8.58 nM. GRP78 is a crucial endoplasmic reticulum (ER) lumen chaperone protein that plays a pivotal role in promoting protein folding and facilitating the cellular stress response (Hendershot, 2004).

3.5. Functions of ISM1

ISM1 is extensively present in various body parts and plays a crucial role in diverse pathophysiological processes including metabolism, immune system functioning, tumorigenesis, cell proliferation, endothelial cell permeability and physiogenesis.

3.5.1. ISM1 in Metabolism

ISM1, an adipokine, plays a pivotal role in body metabolism, diabetes mellitus, and fatty liver disease. It enhances glucose uptake in adipocytes and in muscle cells, inhibits lipogenesis, and boosts protein synthesis in hepatocytes in a way that is independent of insulin (Jiang *et al.,* 2021). Adipokines, which have key functions in the body, act locally on adipose tissues and have an endocrine function, regulate the growth, metabolism, and development of distant organs such as the heart/lungs; bones; pancreas; brain, and liver (Gao *et al.,* 2014; Hu *et al.,* 2022).

Jiang and his team's study (Jiang *et al.,* 2021) on mice that overexpressed or had knock-down of ISM1 revealed that with the participation of mammalian target of rapamycin complex 2 (mTORC2), ISM1 contributes in glucose uptake by the phosphoinositide 3-kinase (PI3K)–protein kinase B (Akt) pathway at residues S^{473} and T^{308} and improved insulin-dependent processes in adipocytes and muscle cells by using a distinct receptor tyrosine kinase unrelated to the insulin-like growth factor 1 and insulin receptors (Jiang *et al.,* 2021; Liang et al., 2024). ISM1 also exhibits activation of extracellular signal-regulated kinase (ERK) (Jiang *et al.,* 2021; Shimizu *et al.,* 2022). Notably, ISM1 triggers Akt phosphorylation which propels glucose transport protein 4 (GLUT4) to move to plasma membrane from cytoplasm, thus initiating glucose uptake. This process is further supported by Akt's suppression of glycogen synthase kinase-3 activation, ultimately increasing glycogen synthesis in adipose tissues (Bhatnagar *et al.,* 2009; Kir *et al.,* 2011; Jiang *et al.,* 2021; Menghuan *et al.,* 2023). Interestingly, ISM1 does not significantly impact other signaling pathways such as protein kinase A and 3-phosphoinositide-dependent kinase 1 (Jiang *et al.,* 2021; Menghuan *et al.,* 2023).

ISM1 may function in lipogenesis via multiple pathways. In hepatocytes, ISM1 suppresses the expression of sterol regulatory element-binding protein-1c (SREBP-1c) and its target genes, such as fatty acid synthase, acetyl-CoA carboxylase, and low-density lipoprotein (LDL) receptor, thereby inhibiting the *de novo* lipogenesis pathway through an unknown receptor. Moreover, ISM1 suppresses the expression of peroxisome proliferator-activated receptor γ coactivator 1β (PGC1β) and carbohydrate response element binding protein (ChREBPβ) (Jiang *et al.,* 2021; Menghuan *et al.,* 2023).

Meanwhile, ISM1 increases protein synthesis in liver and skeletal muscle through the Akt-mTORC1-S6 pathway. Phosphorylated Akt activates mTORC1, which in turn activates $SD^{S235/S236}$, promoting protein synthesis. In contrast, insulin promotes the activation of both protein and lipid synthesis (Jiang *et al.,* 2021; Shimizu *et al.,* 2022). These findings underscore the importance of ISM1 in potential therapeutic interventions for metabolic disorders (Jiang *et al.,* 2021; Shakhawat *et al.,* 2022; Menghuan *et al.,* 2023).

3.5.2. ISM1 in Endothelial Permeability

The endothelial cells, situated on the inner surface of microvessels, are crucial for regulating the exchange of fluid and proteins between the blood and surrounding tissues. This function is essential for maintaining organ homeostasis. Any imperfection in endothelial permeability can result in serious consequences (Shakhawat *et al.,* 2022). ISM1 is widely expressed in brain, lung, and kidney tissues; the released ISM1 enhances endothelial permeability through GRP78 and avβ5 receptors. ISM1-GRP78 complex promotes the permeability of endothelial cells in the lungs. This activation triggers Src-mediated tyrosine phosphorylation of adherens junction proteins, ultimately leading to increased vascular permeability of endothelial cells (Venugopal *et al.,* 2015).

3.5.3. ISM1 in Angiogenesis

Angiogenesis is the development of new blood vessels caused by the multiplication of pre-existing ones. This important process is crucial in organ growth, wound healing, embryo development, and tissue repair in both newborns and adults. The role of ISM1 as an anti-angiogenic factor was first suggested by studies demonstrating that AMOP domain of ISM1 binds with the avB5 integrin and inhibits the formation of capillary networks and VEGF-mediated angiogenesis *in vitro* (Reynolds *et al*., 2002; Xiang *et al.,* 2011; Shakhawat *et al.,* 2022). These studies show that ISM1 exists in two distinct conformational states with disparate effects. Immobilized ISM1 binds to avB5 integrin and activates focal adhesion kinase (FAK), promoting cellular migration and survival. In contrast, soluble ISM1 interaction with avB5 integrin can trigger pro-caspase-8 and this in turn may be cleaved activating caspase 8 to activate further downstream targets like caspase-3 ultimately leading to cellular apoptosis. This suggests that the antiangiogenic functions of ISM1 are compromised in its immobilized form (Zhang *et al.,* 2011; Shakhawat *et al.,* 2022).

Since inhibition of VEGF activation causes the suppression of malignant tumors, ISM1 functions as an inhibitor of malignant tumor. Thus, VEGF inhibition induces apoptosis through the caspase-3 process. This reveals a central role of ISM1 as an inhibitor of in vivo tumor angiogenesis (Yuan *et al.,* 2012).

3.5.4. ISM1 in Apoptosis

ISM1 promotes apoptosis via GRP78 and avB5 integrin-dependent mechanisms. In the extracellular matrix, ISM1 binds to avB5 integrin and inhibits vascular endothelial growth factor (VEGF), ultimately leading to apoptosis. In contrast, ISM1 only weakly prevents serum-stimulated fibroblast proliferation (Liang *et al.,* 2024).

Mobilized ISM1 binds to avß5 receptors and triggers the activation of caspase-8 and caspase-3, ultimately leading to endothelial cell apoptosis (Liang *et al.,* 2024). In contrast, immobilized ISM1 functions as an avß5 integrin multi-protein and a powerful agonist that activates FAK to stimulate endothelial migration, adhesion stability, and cell division (Menghuan *et al.,* 2023; Liang *et al.,* 2024).

Extracellular endothelial and cancer cells express the GRP78 receptor for ISM1. ISM1 binds to GRP78 and is transported into cells through clathrin-dependent endocytosis as an ISM1-GRP78 complex (Menghuan *et al.,* 2023). Na^+/H^+ exchanger regulatory factor 1 (NHERF1) is a cytosolic scaffold protein that functions in Na^+ and H^+ exchanging in endosome. Liang *et al.* (2024) demonstrated that target membrane associated soluble N-ethylmaleimide-sensitive factor attachment protein receptor (t-SNARE) synaptosome-associated protein 25-kDa (SNAP-25) located on the surface of the mitochondria. After interacting of ISM1-GRP78 complex to NHERF1, they transport to the mitochondria and interact with SNAP25 on the mitochondrial surface (Liang *et al.,* 2024). Once inside the cells, this complex interacts with AAC on the inner mitochondrial membrane and disrupts the function of the ADP/ATP carriers on the inner membrane, blocking ATP transport from the mitochondria to the cytosol and initiating the apoptotic process (Wang *et al.,* 2019; Liang *et al.,* 2024). In addition, a cyclic peptide BC71 in the ISM1 AMOP domain is thought to be a pro-apoptotic ligand that binds to GRP78, then activates p53 and caspase-8, thus causing apoptosis (Menghuan *et al.,* 2023).

3.5.5. ISM1 in Hematopoiesis

The process of generating new blood cells in the bone marrow is called hematopoiesis, which is a critical process. Hematopoietic stem cells (HSCs) undergo replication and specialized differentiation to produce their progenitor cells (Menghuan *et al.,* 2023; Chapman *et al.,* 2024). This process begins in the liver during fetal development and continues in the bone marrow in adulthood. Alternatively, hematopoiesis can occur in the spleen, liver, and lungs in pathological conditions of fatigue or inflammation; this is so-called extramedullary hematopoiesis. For example, the lungs of mice are recognized as active hematopoietic nests for platelets, lymphoid as well as myeloid lineages (Rivera-Torruco *et al.,* 2022).

A convincing report by Berrun *et al.* (2018) found that ISM1 is required for HSCs to generate their progenitors in zebrafish. ISM1 efficiently promotes neutrophil/macrophage/erythrocyte production. Despite this, the depletion of ISM1 resulted in a significant decrease in blood cell numbers (Berrun *et al.,* 2018; Liang *et al.,* 2024). Consequently, treatment with ISM1 can significantly promote the preservation of these important cells via the exogenous administration of ISM1 (Liang *et al.,* 2024).

ISM1 has been found in lung stromal niche precursor cells including mesenchymal progenitors (MSC), endothelial progenitor cells (EPCs) and hematopoietic stem and progenitor cells (HSPCs) (Rivera-Torruco *et al.,* 2022). Furthermore, ISM1 has been identified in the LSK cell fraction as well and enhances the enriched capacity of differentiation into lung cells (Rivera-Torruco *et al.,* 2022; Menghuan *et al.,* 2023). These findings demonstrated the significance of ISM1 in hematopoiesis and its expression in various lung cells (Rivera-Torruco *et al.,* 2022; Menghuan *et al.,* 2023).

3.5.6. ISM1 in Immunity

The body's immune system is a sophisticated defense network, comprising the innate and adaptive subsystems. These two crucial systems work in tandem, tirelessly combating any harmful invaders that threaten the body's equilibrium (Brandes *et al.,* 2019; Menghuan *et al.,* 2023). The innate immune system boasts natural killer cells and phagocytes, serving an initial defense against a wide array of pathogens. Conversely, the adaptive immune system deploys antibodies specifically tailored to target previously encountered microbes or pathogens (Brandes *et al.,* 2019; Menghuan *et al.,* 2023). In a study, ISM1 has been revealed to be intricately linked to classical immune signaling pathways such as TGF-β, interleukin 6 (IL6), Janus kinase (JAK)/ signal transducer and activator of transcription 3 (STAT3), interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α)/nuclear factor kappa B (NF-κB), and IL2)/(STAT5). These pathways exert profound effects on crucial immune components, including Treg cell infiltration, programmed death-ligand 1 stability, and CD8+ T cell depletion (Menghuan *et al.,* 2023). Furthermore, the recombinant ISM1 suppresses the replication of the grass carp reovirus by downregulating the expression of vp5, the gene responsible for the virus's outer capsid protein. This inhibition occurs in both in vitro and in vivo settings. Additionally, ISM1 promotes the expression of TANK binding kinase 1 and the interferon regulatory factor 3 pathway, leading to heightened expression of interferon genes and antigens. As a result, it effectively curbs the development of lesions induced by the grass carp reovirus (Liang *et al.,* 2024). This unparalleled defense mechanism augments the body's antiviral response, effectively thwarting viral replication (Li *et al.,* 2021; Menghuan *et al.,* 2023). ISM1 exhibited significant expression in T and NK immune cells of the lung (Valle-Rios *et al.,* 2014; Menghuan *et al.,* 2023), which argues the pivotal role of lung microenvironment to keep intact a population of ISM1+ cells. As a result, it can be said that ISM1 stands as a bulwark against viral threats, wielding the power to decisively suppress viral proliferation while bolstering the body's immune defenses.

3.5.7. ISM1 in Aging

Aging is a natural process that gradually disrupts physiological and biological functions, ultimately resulting in organ dysfunction and mortality (Valenzano *et al*. 2006; Liu *et al*. 2015).

In a study (Markofsky & Perlmutter, 1973), a small fish, Nothobranchius Guentheri (*N. guentheri*), was chosen because of its short lifespan and numerous anatomical and histological similarities with mammalian species. This research has revealed that treatment with salidroside and targeted radiofrequency (TR) therapy can effectively delay age-related biomarkers in *N. guentheri* fishes, potentially extending their lifespan by overcoming the reduction of ISM1. This suggests that ISM1 serves as a reliable biomarker for measuring the "rejuvenated" age of *N. guentheri* fish following treatment with salidroside and TR.

Reactive oxygen species (ROS) are commonly produced as by-products of oxidative phosphorylation and can bind to cellular components, disrupting normal cellular functions. Oxidative stress, resulting from an imbalance between ROS generation and detoxification, can accelerate the aging process. ISM1 exerts its rejuvenation activity by enhancing the antioxidant system (Valenzano *et al.* 2006; Liu *et al.* 2015). It showed that when *rIsm1* exerts its rejuvenation activity, it stimulates the activation of the antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) and reduces oxidative stress. In turn, slow down the protein and lipid oxidation and arguably senescence-associated β-galactosidase (SA-β-Gal) and lipofuscin development, and ultimately result in prolonged lifespan of *N. guentheri*. This study is the first to point out that ISM1 is an age-related biomarker, which decreases with age in humans, fish and mice (Li *et al.,* 2022). A study conducted by Hu *et al.* (2024) confirmed that ISM1 efficiently retards aging-related cardiac dysfunction by enhancing glycolysis and activating sirtuin-1 (SIRT1) deacetylase by raising GLUT4-mediated glucose uptake. In summary, ISM1 improves the quality of life and suppresses aging-related cardiac disease in the elderly.

3.5.8. ISM1 in Kidney Development

Studies have found that ISM1 is widely expressed in the metanephric mesenchyme and ureteric epithelium, and it plays a part in transcriptional maintenance and branching morphogenesis in early kidney development (Gao *et al.,* 2023). ISM1 has three receptors located in the mesenchyme cells: α8β1, ephrin-β1, and plexin-β2, all of which are located in the mesenchyme cells of the developing kidney. ISM1 interacts with α8β1 to stimulate FAK, Akt, and ERK phosphorylation, cell-to-cell adhesion, and mesenchyme condensation. On the other hand, ISM1 binds to ephrin-β1 and plexin-β2, triggering cell adhesion, cell migration, and cytoskeleton rearrangement in several tissues during kidney development. For this reason, mice deficient for *Ism1* manifested renal agenesis and hypoplasia (Gao *et al,* 2023).

3.6. ISM1 in Some Diseases

3.6.1. ISM1 in Diabetes

Maintaining glucose homeostasis is essential and requires a delicate balance between glucose uptake by adipose tissue, skeletal muscle and heart,

and gluconeogenesis by liver, kidney and gut (Szablewski, 2017). Diabetes is a global health concern. Type 2 diabetic mellitus (T2DM) is a chronic metabolic disorder featured by sustained hyperglycemia originating from insulin resistance (IR) and pancreas β-cell failure, ultimately resulting in declined glucose uptake by the liver, skeletal muscles, and adipose tissues (Wang *et al.,* 2022; Liao *et al.,* 2023; Liang *et al.,* 2024). Hyperglycemia induces multiple microvascular complications such as diabetic nephropathy, diabetic retinopathy, and peripheral sensory and autonomic neuropathy (Wang *et al.,* 2022).

Serum ISM1 levels display a direct correlation with estimated glomerular filtration rate, and its elevation shows the potential to be utilized as a reliable biomarker for anticipating kidney function decline in patients with T2DM (Liang *et al.,* 2024). A recent study has established a connection between serum ISM1 levels and IR as well as early-stage diabetic nephropathy. It was observed that serum ISM1 levels rise with albuminuria in DN patients, aligning with the severity of albuminuria in T2DM patients. However, no significant correlations were found between serum ISM1 levels and IR (Wang *et al.,* 2022).

In diabetic conditions, the release of insulin by pancreatic cells is intended to increase glucose uptake. However, excessive insulin or insulin secretagogues can lead to lipogenesis in the liver and other tissues that ultimately culminates in severe IR and non-alcoholic fatty liver disease (NAFLD) (Röder *et al.,* 2016; Shimizu *et al.,* 2022). Research has verified that ISM1 plays a crucial role in regulating glucose uptake, enhancing insulin sensitivity, and suppressing lipogenesis through the independent activation of the PI3K/Akt pathway, irrespective of insulin and insulin-like growth factor (IGF) receptors (Jiang *et al.,* 2021). Elevated serum ISM1 levels were linked to a reduced risk of diabetes, indicating that ISM1 acts as a protective factor against diabetes in the population (Wang *et al.,* 2022).

Serum ISM1 levels were directly affected in T2DM, no significant changes were observed in patients with diabetes-associated NAFLD, suggesting that ISM1 serves as an independent marker for diabetes but not for diabetes-associated NAFLD (Wang *et al.,* 2022). However, another study has found that serum ISM1 levels were significantly higher in patients with T2DM compared to non-diabetics, this emphasizes that ISM1 levels may vary depending on species and nutritional status (Liao *et al.,* 2023). Furthermore, one study has tested the elevation of serum ISM1 levels among obese individuals, the study has strikingly demonstrated a significant increase in ISM1 levels in obese females compared to lean females, however, no significant differences in ISM1 levels have been observed among males (Liao *et al.,* 2023). A study on T2DM observed that serum ISM1 levels were elevated in obese boys but not in girls (Ruiz-Ojeda *et al.,* 2023). Another study has observed that ISM1 is significantly linked to isolated post-challenge hyperglycemia (IPH); this

association was significantly found in men. In men, elevated levels of ISM1 were found to be significantly and independently associated with a lower risk of IPH, whereas decreased levels of ISM1 were noted in men with diabetes and IPH. No association between ISM1 and the risk of IPH in women was found (Fan *et al.,* 2024). A research demonstrated that patients with coexisting metabolic-associated fatty liver disease, metabolic syndrome and T2DM displayed escalating ISM1 levels compared to control group (Lei *et al.,* 2024). Serum ISM1 levels are directly related to body mass index, fasting insulin, total cholesterol, LDL-cholesterol, uric acid, aspartate aminotransferase and alanine aminotransferase, whereas they are inversely related to age and high-density lipoprotein cholesterol levels (Lei *et al.,* 2024). Moreover, a study found that serum ISM1 levels were higher in males than in females. Additionally, ISM1 levels were higher in obese patients than in lean patients and this conclusion was also true for both males and females (Lei *et al.,* 2024).

Serum ISM1 levels were not related to diabetic sensorimotor peripheral neuropathy (DSPN), which is a debilitating condition caused by continuous exposure to insulin resistance and high blood sugar levels, and affects a staggering 30% of individuals living with diabetes (Liao *et al.,* 2023).

According to the above mentioned studies, it can be summarized that ISM1 is crucial in protecting against diabetes and diabetic nephropathy, but it does not manifest any effect on insulin resistance in patients with T2DM. Additionally, serum ISM1 levels show no alterations in patients with diabetes-associated NAFLD and DSPN.

3.6.2 ISM1 in Lung Disorders

ISM1 is highly expressed in the mouse lung, it is mainly related to anti-inflammatory actions and helps maintain lung homeostasis (Nguyen *et al.,* 2022).

Lung diseases affect millions of people in the U.S. alone, making them prevalent medical conditions worldwide. The primary causes of these diseases are smoking, infections, and genetics (Hoffman, 2022). In chronic obstructive pulmonary disease, which is a serious inflammatory condition, the number of inflammatory alveolar macrophages (AM) and alveolar macrophage elastase (MMP-12) were found to be increased (Uwagboe *et al.,* 2022). ISM1 plays a vital role in reducing lung inflammation by binding to csGRP78 high AMs, targeting apoptosis, preventing emphysema progression, and preserving lung function. Conversely, a study found that *Ism1* knockout mice (*Ism1-/-* mice) resulted in an elevated number of csGRP78-high AM and accompanied MMP-12, leading to emphysema and progressive lung inflammation (Lam *et al.,* 2022).

Asthma is a respiratory disroder characterized by airway inflammation, airflow obstruction, and bronchial hyperresponsiveness. Shortness of breath, cough, and wheezing are prominent symptoms in these patients (Hashmi & Cataletto, 2024). In a study, it was expressed that ISM1 plays a crucial role in maintaining the integrity of the lung trachea and in suppressing lung inflammation and airway hyperresponsiveness in mice with house dust mite (HDM)-induced asthma. In the lung, ISM1 potentially stimulates the secretion of adiponectin from type 2 alveolar epithelial cells. The secreted adiponectin enhances apoptotic cell efferocytosis by AMs both *in vitro* and *in vivo*, which attenuates HDM-induced airway inflammation. Therefore, the deficiency of ISM1 results in delayed clearance of dead eosinophils by AMs, leading to exacerbated necroptosis and lung inflammation (Tee *et al.,* 2023).

The increased levels of ISM1 in alveolar epithelial cells type II (AECII) can instigate hypoxia, resulting in hyperpermeability. This hyperpermeability of pulmonary microvascular endothelial cells induced by hypoxia can lead to high-altitude pulmonary edema (HAPE) and various pathological conditions in the lung (Shakhawat *et al.,* 2022).

3.6.3. ISM1 in Cancer

Several studies have demonstrated a strong correlation between ISM1 and the progression of tumorigenesis. ISM1 has been shown to regulate the invasion and migration of tumor cells such as melanoma, hepatocellular, colorectal and breast cancinomas (John *et al.,* 2006).

Non-coding RNA (ncRNA) despite not encoding proteins, these remarkable RNA molecules possess vital information and exert specific, profound influences, they are pivotal players in physiological and pathological processes, wielding significant influence as both drivers of oncogenesis and suppressors of tumorigenesis in numerous cancer types (John *et al.,* 2006). Particularly noteworthy are microRNA (miRNA) and circular RNA (circRNA), which stand out as the foremost impactful varieties of ncRNA (Menghuan *et al.,* 2023; Liang *et al.,* 2024). Wang and colleagues (2019) have suggested that ISM1 is involved in hepatocellular carcinoma. The circular RNA hsa_circ_0091570 promotes the activation of miR1307, which in turn is essential for activating the expression of ISM1, leading to decreased cancer cell proliferation and migration. So that downregulation of hsa_circ_0091570 was observed in hepatocellular carcinoma (HCC) and can function as competitive endogenous RNA (ceRNA) by interacting with miR-1307 to regulate ISM1 expression, thus playing a crucial role in HCC progression (Wang *et al.,* 2019). However, another study has found that increased expression of the ISM1 gene may lead to colon adenocarcinoma. Inhibiting the activation of the ISM1 gene by miR-1307–3p can suppress the Wnt/β-catenin signaling pathway, inhibiting proliferation and promoting apoptosis of colon adenocarcinoma cells (Zheng

et al., 2019). Additionally, Wu and colleagues have observed upregulation of ISM1 in colorectal cancer cell (CRC) tissues and in multiple cancer-related pathways (Wu *et al.,* 2021). Additionally, another study has stated that ISM1 is overexpressed in the blood and tissues of CRCs. Under hypoxic conditions, CRCs promote the overexpression of ISM1 via hypoxia-inducible transcription factor. The released ISM1 interacts with the epidermal growth factor receptor (EGFR), initiating tumor development, migration, and invasion. Additionally, ISM1 binds to Y-box binding protein 1 (YBX1), promotes YBX1 phosphorylation, and triggers its transcriptional activity on EGFR expression. Thus, ISM1 enhances the development and metastasis of colorectal cancinoma through activation of the EGFR signaling pathway (Zhou *et al.,* 2024).

ISM1 significantly enhances the invasiveness and migration of coronary adenocarcinoma by decreasing E-cadherin levels and promoting epithelial-mesenchymal transition (Liang *et al.,* 2024). ISM1 could be a predictor in prognosis of lobular breast cancer. In *ISM1* gene promoter region, the clusters of methylation were detected (Suman *et al*., 2021). The recombinant ISM1 injection stimulated cell apoptosis in breast carcinoma and melanoma, and decreased tumor vascularization and cell proliferation via the cyclic peptide BC71 of ISM1 (Liang *et al.,* 2024). In summary, ISM1 could be a potential candidate for cancer treatments. Moreover, long ncRNAs have a significant impact on the progression and metastasis of different types of cancers. Particularly, the overabundance of lncRNA H19 in gastric cancer tissues has been noted. This abundance leads to the binding of ISM1 to lncRNA H19, resulting in the upregulation of ISM1, which in turn enhances the carcinogenic and metastatic potential of gastric cancer (Shakhawat *et al.,* 2022).

4. ISM2

Ism2 gene, situated on chromosome 14q24.3 in humans. This influential gene encodes a secreted protein with TSR1 and AMOP domains, and it is 63.9 kDa. (Rossi *et al.,* 2004; Li *et al.,* 2021; Shakhawat *et al.,* 2022). Notably, ISM2 harbors as well high-quantum motifs "EPQ" and "WSPW", related to sugar binding specificity determination and TGF-B activation forces (Table 2) (Lawler & Hynes, 1986; Dawson *et al.,* 1997; Rega *et al.,* 2008; Abderrazak *et al.,* 2018).

ISM2 is primarily expressed in the placenta. It is linked to choriocarcinoma and preeclampsia (Martinez *et al.,* 2020; Liang *et al.,* 2024). In preeclampsia, ISM2 is upregulated, leading to an increase in anti-angiogenic factors and a decrease in angiogenic factors, potentially contributing to the development of preeclampsia (Liang *et al.,* 2024).

Conversely, ISM2 has been associated with an increased occurrence of choriocarcinoma, as it leads to a decrease in anti-angiogenic factors and an increase in angiogenic factors in patients (Liang *et al.,* 2024). The high

expression of ISM2 in choriocarcinoma patients, as well as its moderate expression in lung and prostate adenocarcinoma, suggests that ISM2 may serve as a potential diagnostic or prognostic marker in these conditions (Shakhawat *et al.*, 2022). Further research is needed to determine whether ISM-2 can be used as a biomarker for preeclampsia and choriocarcinoma.

The role of ISM2 in metabolism has yet to be fully explored in scientific studies.

Table 2. *The important motifs of ISM2 domains*

Domain	Motif	Function
TSR1		
	EPQ	Carbohydrate binging
	WSPV	TGF-β activation
AMOP		
	WSRL	Inducing of autophagy
	KGD	αIIbβ3 binding in platelets
		Cell adhesion
		Tumor metastasis

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Chapter 10

EFFECTIVENESS OF NEURODYNAMIC MOBILIZATION TECHNIQUE ON DELAYED ONSET MUSCLE SORENESS-ASSOCIATED MUSCLE DAMAGE AND INFLAMMATORY BIOMARKERS

Uğur SÖZLÜ[1], Selda BAŞAR[2], Rabia ŞEMSİ[3], Esedullah AKARAS[4], Aylin SEPİCİ DİNÇEL[5]

1 Dr. Lecturer Uğur SÖZLÜ. Department of Physical Therapy and Rehabilitation, Health Science Faculty, Gaziosmanpasa University, Tokat Turkey. sozluugur@gmail.com ORCID No: 0000-0001-5171-161X

2 Prof. Dr. Selda BAŞAR. Department of Physical Therapy and Rehabilitation, Health Science Faculty, Gazi University, Ankara, Turkey. seldabsr@yahoo.com ORCID No: 0000-0002-1433-4349

3 MSc Rabia ŞEMSİ. Department of Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Turkey. rabiasemsi2010@gmail.com ORCID No: 0000-0002-8477-5537

4 Dr. Lecturer Esedullah AKARAS. Department of Physical Therapy and Rehabilitation, Health Science Faculty, Erzurum Technical University, Erzurum, Turkey. esed87@gmail.com ORCID No: 0000-0002-0305-4632

5 Prof. Dr. Aylin SEPİCİ DİNÇEL. Department of Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Turkey. asepicidincel@gmail.com ORCID No: 0000-0001-5847-0556

INTRODUCTION

The concept of administering mechanical treatment to brain tissue is not novel. Breig and his colleagues created this approach in 1978, which Butler subsequently characterized as the 'Neural Tension Test' to measure the nervous system's physical sufficiency for movement and nerve transmission (DS Butler & Jones, 1991). Shaclock and colleagues developed the more sophisticated term 'Neurodynamics' to help explain physiology, pathology, and pathomechanics in the mechanical therapy of the nervous system (DS Butler & Jones, 1991; D. S. Butler, 2000; Shacklock, 1995).

Neurodynamics refers to all of the links between the morphology, biomechanics, and physiology of the neural system (D. S. Butler, 2000; Shacklock, 2005a; Sunderland, 1990). Neural mobilization techniques are a collection of manual procedures that allow for the mobility of neural tissue and structures around the neural system (Dwornik, Białoszewski, Korabiewska, & Wroński, 2007). It is frequently used nowadays to evaluate and enhance the mechanical and neurophysiological integrity of peripheral nerves (Shacklock, 1995).

Neurodynamic mobilization (NM) of peripheral nerves is favored to boost axonal transport, improve nerve conduction, and lower pressure inside the nerve, therefore enabling improved blood flow to the nerve (David Butler & Gifford, 1989; Shacklock, 2009). The goal is to restore dynamic equilibrium between the nerve and surrounding tissues while also improving functioning by promoting regeneration and repair of the damaged nerve via increased blood flow (Dwornik et al., 2007; Ellis & Hing, 2008). Neurodynamic mobilization also attempts to restore joint flexibility, reduce pain, enhance blood supply to neural tissue, decrease edema in neural tissue, offer axonal transfer, lower sympathetic tone, and control aberrant neuromechanical activities (D Butler, 1991; DS Butler & Jones, 1991; D. S. Butler, 2000; Coppieters & Butler, 2008; Dwornik et al., 2007; Ellis & Hing, 2008). Neurodynamic mobilization techniques are a type of manual treatment performed to neural structures employing numerous joint motions and positions. They include combined movements of proximal-distal segments and repeated movements of segments (Shacklock, 2005a). It may be used in two ways: stretching (displacing nerve ends in the opposite direction) and sliding (displacing nerve endings in the same direction) (Coppieters & Alshami, 2007; Coppieters & Butler, 2008; Coppieters, Hough, & Dilley, 2009).

There are two major processes underlying the mechanical and physiological impacts of Neurodynamic mobilization (DS Butler & Jones, 1991; Coppieters & Butler, 2008). The first of them is the pumping and milking effects that occur as a result of repeated motions of the nerve tissue (Coppieters & Butler, 2008). Millesi et al. discovered that the pumping and milking action

enhances axoplasmic flow in the nerve and surrounding connective tissues, facilitating the transmission of local inflammatory agents (Coppieters & Butler, 2008; Gilbert et al., 2015). The second mechanism of action is that neurodynamic mobilization increases the activation of glial cells (Santana, Fernandes de Oliveira, Medrado, & Nunes, 2015). Microglia and astrocytes, subtypes of glial cells, are the fundamental cells that allow communication between the immune system and the central nervous system, and they play a role in the development of inflammatory processes (David, Greenhalgh, & Kroner, 2015; Milligan & Watkins, 2009). These are also known as the central nervous system's 'permanent immune system cells'. In the healthy phase, when there is no damage, they cause a sterile inflammation at the base level and induce synaptic plasticity in the brain. The goal is to keep the central nervous system aware against any injury that may occur with the growing plasticity and learning (David et al., 2015). Neurodynamic mobilization is supposed to activate nerves and disclose the plasticity property of glial cells, therefore helping to healing (Santana et al., 2015; Santos et al., 2012).

Delayed-onset muscler soreness (DOMS) is a clinical syndrome in which metabolites and byproducts of tissue damage temporarily accumulate in the body as a result of exercise-induced muscle injury (Brown, Chevalier, & Hill, 2010; Cheung, Hume, & Maxwell, 2003). DOMS is classified as a first-degree muscular strain and/or microtear that does not include a partial (grade II) or complete (grade III) muscle tear (Cheung et al., 2003). DOMS is defined as a type 1 strain injury (Armstrong, 1984; Cheung et al., 2003). Symptoms include discomfort, sensitivity to palpation and/or movement, reduced range of motion, strength, and performance, and edema (Cheung et al., 2003). The resulting pain and discomfort often begins in the distal region of the muscle (near the muscle-tendon junction), becomes noticeable in 24-48 hours, and reaches its peak levels within 24-72 hours (Guilhem, Cornu, & Guével, 2010; Isner-Horobeti et al., 2013).

Studies have indicated that the neurodynamic mobilization approach is beneficial in enhancing the quality of life of people with neuropathic pain and other pain disorders (Anandkumar, 2012; Dwornik et al., 2007; Ellis & Hing, 2008; Oskay et al., 2010; Véras et al., 2012; Villafañe, Cleland, & Fernandez-De-Las-Penas, 2013). Studies have demonstrated that the neurodynamic mobilization approach promotes performance, range of motion, flexibility, and pressure pain threshold, not just in patients but also in healthy persons (Aksoy, Kurt, Okur, Taspınar, & Taspinar, 2020; Basson et al., 2017; Beneciuk, Bishop, & George, 2009; Castellote-Caballero et al., 2013; Herrington, 2006; Kim, Cha, & Ji, 2016; Shacklock, 2005b; Sharma & Cleland, 2016). However, current neurodynamic studies demonstrate substantial heterogeneity in treatment durations, and there is no uniformity of therapy dose (Allison, Nagy, & Hall, 2002; Baysal et al., 2006; Bialosky et al., 2009; Cleland, Childs,

Palmer, & Eberhart, 2006; George, 2002; Sahar, 2011; Schäfer, Hall, Müller, & Briffa, 2011; Tal-Akabi & Rushton, 2000). To the best of our knowledge, there have been no published trials comparing therapy durations. Treatment dose is often modified based on patient response and clinical experience (Baysal et al., 2006; Cleland et al., 2006; Coppieters, Stappaerts, Wouters, & Janssens, 2003; George, 2002; Schäfer et al., 2011). As a result, neurodynamic mobilization promotes neuronal plasticity, decreases the dynamic sensitivity of the nervous system, and increases blood flow, alleviating pain and facilitating bodily mobility (D. S. Butler, 2000; Shacklock, 2005b). The aim of this study is to investigate the effects of the neurodynamic mobilization technique on delayed onset muscle soreness-associated muscle damage and inflammatory biomarkers.

METHODS

Participants

This research was designed as a pilot study. At the beginning of the study, 10 male participants were assessed. Five individuals were excluded because they did not match the eligibility requirements. Finally, 7 subjects took part in the study (Figure 1).

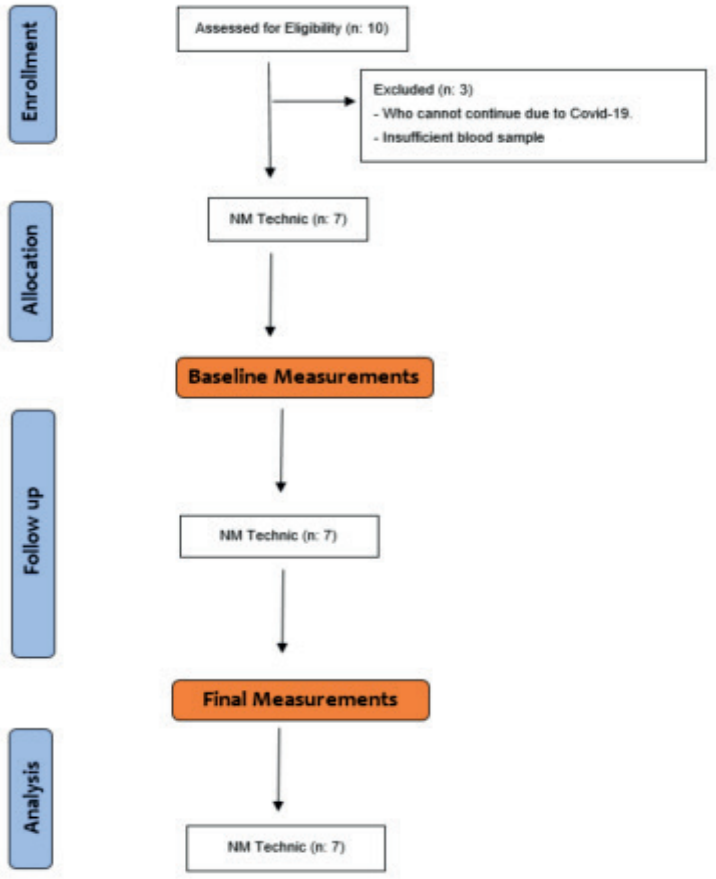

Fig.1. Participant flowchart

The criteria for inclusions were as follows: 1) between the ages of 20 and 32; 2) male; and 3) inactive. Individuals with neurological and/or perception problems, any systemic disease, limitations in any of the knee flexion or extension movements, lower extremity pain, injury or surgery history, those using anti-inflammatory and/or analgesic drugs at least 12 hours before the evaluations, those smoking and/or drinking alcohol, and those with insufficient blood samples were excluded from the study. Each subjects provided written informed consent. Necessary permissions were obtained from the University Ethics Committee for this pilot study, which was conducted to investigate the effects of the neurodynamic mobilization technique on muscle damage and inflammation biomarkers.

Study Design

In this study, which was designed as a pilot study, firstly the demographic information of the individuals invited to the study was questioned and

recorded. Individuals who met the inclusion criteria were assigned to the neurodynamic mobilization group. In order not to affect the measurements, individuals were told not to take any analgesic, anti-inflammatory drugs and caffeine-containing foods from the beginning of the evaluations until the end of the treatment, and not to apply anything to the area where muscle damage was created (such as movement, heat or ice application). The study was carried out in three stages, as stated below, by taking care to ensure that all applications and measurements were made at the same time of day for each individual, by the same researcher and on the dominant lower extremity.

Stage 1: Initial assessments were completed, and blood samples were collected for biochemical analysis. Following that, the neurodynamic mobilization approach was used for three weeks. At the end of the third week, three days were allowed to pass to eradicate the acute impact of neurodynamic mobilization.

Stage 2: Delayed Onset Muscle Soreness Induction Protocol was implemented.

Stage 3: Immediately after DOMS, initial assessments were repeated and blood samples were taken for biochemical analyses.

Measurements

Blood sampling and analysis

A total of 7 mL blood samples were taken from the antecubital veins of the dominant arms of the individuals, 5 mL (with jelly - yellow cap) and 2 mL (with ethylene diamine tetra acetic acid - purple cap). The blood samples were collected and distributed to 1.5 mL Eppendorf tubes as 500-1000 µL. These samples were then stored at -80°C until the day they were analyzed.

Afterwards, serum CK and AST measurements of muscle damage parameters were studied in the Biochemistry Laboratory using Beckman Coulter brand Au680 model biochemistry autoanalyzer with Beckman Coulter brand kits. IL-10 and IL-1β levels of inflammatory biomarkers were measured by solid phase sandwich ELISA method using Human IL-10 Elisa Kit (USCN, Lot: L201013958) and Human IL-1β Elisa Kit (USCN, Lot: L201013957) by expert personnel in accordance with the manufacturer's instructions.

Neurodynamic mobilization technique

Femoral nerve neurodynamic mobilization techniques were applied by a trained physiotherapist with an internationally valid certificate (from Neuro Orthopedic Institute, Australia). The individual was laid on his side with the non-dominant leg underneath. The individual was asked to grasp the lower knee with his hand and pull it towards himself and at the same time to extend his head. In this position, the practitioner stabilized the pelvis with one hand

and grasped the upper and flexed knee with the other hand and made the hip fully extend. After waiting for 3 seconds in this position, the starting position was returned. 3 seconds were waited for the next repetition. This procedure was applied in three sets of 10 repetitions, 3 days a week and for 3 weeks. A 2-minute rest period was given between sets (Woods, Bridge, Nelson, Risse, & Pincivero, 2004).

Delayed Onset Muscle Soreness Protocol

An isokinetic dynamometer (Cybex Humac Norm Testing and Rehabilitation System, CSMI, USA) was used to create a DOMS. Individuals were seated on the isokinetic dynamometer with their trunks at 80° flexion. Their backs were supported and their pelvises were fixed with a belt. The rotation center of the dynamometer was placed in line with the center of the dominant knee joint. The resistance pad was connected to the upper part of the ankle joint. Individuals were asked to hold the handles on the sides of the seat of the device during the test. The knee flexion range of motion was set to 35°- 95° and the speed was set to 30°/sec. Individuals were asked to perform 10 repetitions of submaximal eccentric knee extension for warm-up purposes. Then, 30 sets of maximal eccentric knee extensions with 10 repetitions were performed in the same position and angular speed to create a DOMS. The protocol was completed in an average of 30 minutes, with 20 seconds of rest between sets. During the test, the individual was instructed to extend the knee while the lever arm of the isokinetic dynamometer was going up (passive mode, no force required) and to show maximum resistance (eccentric mode) while the lever arm was going down. In order to get maximum performance, the individual was verbally encouraged and visual feed-back was provided on the device screen (Akalin et al., 2002; D. MacIntyre, Reid, Lyster, Szasz, & McKenzie, 1996; D. L. MacIntyre, Sorichter, Mair, Berg, & McKenzie, 2001).

Data Analysis

The data from the patient and control groups were analyzed using the ''Statistical Package for Social Sciences'' (SPSS) Version 22.0 (SPSS Inc. Chicago, II, USA). To evaluate if numerical variables were regularly distributed, the Shapiro-Wilk test was used, along with suitable graphical approaches. Descriptive statistics for numerical variables were given as mean and standard deviation (X ± SD). The Wilcoxon Test was used to examine changes in muscle injury and inflammatory biomarker measures. Statistical significance was considered at $p < 0.05$.

RESULTS

Characteristics of the subject

Participants' age, body mass index and dominant side information are shown in Table 1.

Table 1. *Demographics of participants'*

	NM (n=10) X ± SD
Age (year)	25.4 ± 3.9
BMI (kg/m²)	22.2 ± 2.4
Dominant side (Right/Left)	10/0

Biomarkers of muscle damage and inflammation

Measurements of muscle damage biomarkers such as Serum creatine kinase (U/L) and Aspartate aminotransferase (U/L), inflammation biomarkers such as Interleukine -10 (pg/mL) and Interleukine -1β (pg/mL) are examined in Table 2. In the analysis, it was seen that the neurodynamic mobilization technique applied for three weeks did not cause any statistically significant change in either muscle damage or inflammatory biomarkers (p>0.05).

Table 2. *Changes in biomarkers of muscle damage and inflammation*

	Before NM X ± SD	After NM X ± SD	p*
Serum creatine kinase (U/L)	155±97	278±209	0.059
Aspartate aminotransferase (U/L)	20±4	29±19	**0.037**
Interleukine -10 (pg/mL)	59±41	153±65	**0.005**
Interleukine -1β (pg/mL)	151±89	216±169	0.059

*Wilcoxon Test

DISCUSSION

As a result of this study, which was conducted to investigate the effects of neurodynamic mobilization technique on DOMS related muscle damage and inflammation biomarkers, it was seen that neurodynamic mobilization technique did not cause any change in Serum creatine kinase (U/L) and Interleukine -1β (pg/mL) levels. An increase in Aspartate aminotransferase (U/L) and Interleukine -10 (pg/mL) levels was observed.

The applied neurodynamic mobilization technique was applied in two ways as stretching or sliding. However, there is no consensus in the literature regarding the selection of the neurodynamic mobilization technique to be applied. Since the nerve sliding technique is more painless and tolerable than the stretching method, it is preferred in patients and individuals with acute pain, while the stretching technique is used in healthy individuals and individuals with chronic pain (Coppieters & Butler, 2008). It has also

been shown that the stretching technique is more effective than the sliding technique in parameters such as pressure pain threshold, muscle strength, ROM and performance (Gamelas et al., 2019; Martins et al., 2019). For this reason, the nerve stretching technique was preferred in our study.

Some studies have been conducted investigating the effects of neurodynamic mobilization on muscle damage biomarkers (Kim et al., 2016; Romero-Moraleda et al., 2017). Kim et al. applied therapeutic ultrasound and median nerve mobilization to the biceps brachii muscle where muscle damage was created and reported that muscle damage symptoms such as pain, pressure pain threshold, and lactate level occurred less in the neurodynamic mobilization group (Kim et al., 2016). Romero et al. applied the femoral nerve neurodynamic mobilization technique after muscle damage caused by high jumping and recorded a healing effect on muscle damage parameters (Romero-Moraleda et al., 2017). In our study, while the level of Aspartate aminotransferase (U/L), one of the biomarkers of muscle damage, increased, the serum creatine kinase level did not change. We think that this increase is due to the applied DOMS protocol and that the neurodynamic mobilization technique has no effect. We think that the results obtained from these studies may differ due to both the differences in application techniques and the small number of studies.

Nosaka and colleagues suggested that neural adaptation positively regulates the workload distribution among muscle fibers, thereby reducing the stress per fiber (Nosaka & Clarkson, 1995). Based on this information, in our study, it was aimed to create an effect on muscle damage parameters by increasing the adaptation and capacity of the nerves with the neurodynamic mobilization technique applied for 3 weeks. According to the results we obtained, it was found that the neurodynamic mobilization technique applied for three weeks did not create any positive or negative changes in any of the muscle damage biomarkers.

Considering these results, it is thought that the neurodynamic mobilization technique we applied increased neural adaptation and distributed the contraction stress over a larger number of muscle fibers, limiting myofibrillar disruption and therefore the release of muscle damage parameters. However, as far as is known, the fact that there is no study in the literature examining the effects of the neurodynamic mobilization technique applied to healthy individuals on muscle damage biomarkers has limited the comparability of the results we obtained. In this respect, we believe that our study has contributed significantly to the literature. We believe that biopsy or animal studies examining the effectiveness of neurodynamic mobilization on muscle damage markers at the tissue level will clarify the issue.

Cytokines regulate immune and inflammatory processes, including inflammation, cell growth, and systemic responses to injury (Pedersen et al., 2001). The most important muscle damage parameters are IL-10 and IL-1β. Cytokines are synthesized and released in peripheral immune cells as well as in glial cells (microglia and astrocytes), which are the immune cells of the central nervous system (Colburn, Rickman, & DeLeo, 1999; Rittner, Brack, & Stein, 2002). However, it was stated in a review by Santana et al that the neurodynamic mobilization technique may increase the activation of glial cells responsible for the production of cytokines (Santana et al., 2015). In our study, it was determined that the neurodynamic mobilization technique applied for three weeks did not create a significant difference in terms of IL-1β level except IL-10.

CONCLUSION

As a result of neurodynamic mobilization application, it was observed that there were no significant changes in serum CK and IL-1β values after DOMS. However, AST and IL-10 levels were found to increase. Therefore, it was concluded that further studies are needed to obtain more definitive results.

Conflict of Interest and source of funding statement

The authors declare that they have no conflict of interest. This work has been supported by GAZİ University Scientific Research Projects Coordination Unit (under grant number: 47/2020–02).

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