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Editor

Nihal Inandiklioglu

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FOREWORD

Dear Readers,

We take pride in presenting our book titled "**Current Studies in Basic Medical Sciences**" comprising a total of 8 chapters prepared by experts in the field. Each chapter covers contemporary topics based on current research and the latest findings. The authors of each section have skillfully blended theoretical knowledge with detailed literature support and their own experiences. This book will serve as a valuable resource for academics and students in the field of health who are keen on staying updated with the latest developments.

We extend our gratitude to everyone involved in the preparation of this book and wish you enjoyable reading.

Best regards,
Assoc. Prof. Dr. Nihal İNANDIKLIOĞLU
Editor

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CHAPTER I

BLOOD SUPPLY OF THE CENTRAL NERVOUS SYSTEM

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1. Introduction

Functionally, the central nervous system (CNS) is the body's most metabolically active system. Since the brain tissue does not have its own oxygen and glucose reserve, the metabolic demands of the system are met by glucose and oxygen carried by the blood circulation. Cerebral blood flow (CBF) is 700-850 ml/min in a normal human brain weighing 1350-1500 g. This corresponds to a blood flow of 50-54 ml per minute for approximately 100 grams of brain tissue. Although the brain constitutes only 2% of the body weight, it consumes approximately 12-15% of the blood pumped by the heart per minute and 20% of the oxygen utilized by the whole body. Therefore, the blood supply of the CNS is of paramount importance. Even short-term interruptions in CBF can lead to severe neurological disorders. (1-6) A reduction in blood flow in the cerebral tissue to 25 ml/min results in ischemic penumbra, while a decrease to 8 ml/min causes loss of function in the vast majority of neurons. Loss of consciousness occurs within 10 seconds after cessation of cerebral blood circulation. (2)

Blood circulation is also crucial to meet the metabolic demands of the spinal cord, which is part of the CNS. The blood flow in the spinal cord should normally be 30-35 ml/min per 100 grams of tissue. In the case of a drop in blood pressure or occlusion in the arteries supplying the spinal cord, the cells cannot survive, as oxygen and metabolic needs will not be met. Structurally, the brain tissue is vulnerable to ischemia, and neurological impairments arising from

disturbance in blood supply result in damage to the area supplied by the affected vessel. The severity of the resulting damage varies depending on the duration of ischemia and the size of the affected area. (2)

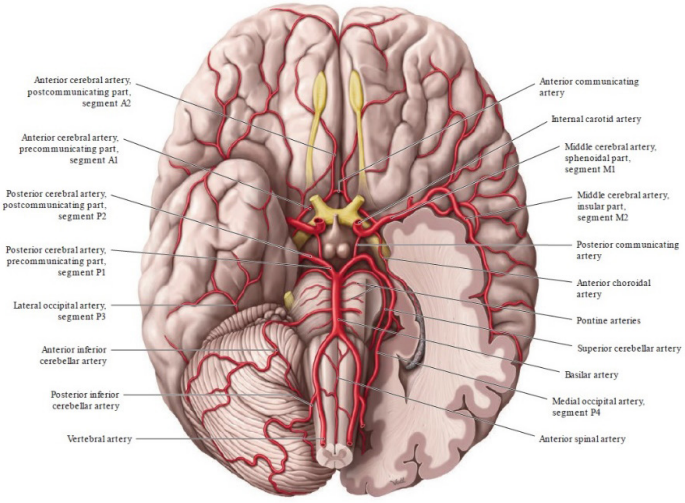
The CNS is primarily supplied by two vascular systems, namely the vertebrobasilar system and the carotid system. The basilar artery and vertebral artery form the vertebrobasilar system, the branches of which supply the occipital lobe, infratentorial region, cerebral trunk, cerebellum, and the spinal cord. On the other hand, the internal carotid artery forming the carotid system supplies all areas in the brain hemispheres except the occipital lobe. Apart from the basilar artery itself, all other arteries supplying the entire brain emerge in pairs, with one artery on the left and right. (1, 2, 4, 7)

2. Vertebrobasilar system

2.1. Vertebral artery

After emerging from the subclavian artery as the first branch, the vertebral artery (VA) passes through the transverse foramina of all cervical vertebrae except the 7th cervical vertebra (C7) and continues its upward course. Once the vertebral artery reaches the suboccipital region, it changes direction medially on the upper border of the atlas. There, it pierces the posterior atlanto-occipital membrane, passes through the foramen magnum and reaches the posterior cranial fossa. Within the lateral cerebellomedullary cistern known as the subarachnoid space, it travels forward, inward and upward, closely neighboring the roots of the hypoglossal nerve until it reaches the bulbopontine sulcus. At this point, below the clivus, the right and left vertebral arteries join to form the basilar artery. The basilar artery extends within the basilar sulcus to the pontocrural sulcus, and then bifurcates into its terminal branches, the left and right posterior cerebral arteries. (1, 2, 4, 7, 8) (Figure 1)

Figure 1. Cerebral arteries (inferior view of the brain)



Source: "THIEME Atlas of Anatomy". (9)

Although variations are frequently observed in each segment of the vertebral artery starting from its origin, pathological conditions such as congenital fenestration, duplication, atresia or hypoplasia may also occur. Occlusions in the proximal part of the VA are unlikely to cause an infarct or other clinical symptoms in the cerebral trunk or cerebellum because the contralateral vertebral artery will continue to supply this region. Occasionally, weakness in the ipsilateral upper extremity and dizziness may occur. However, the clinical course of the pathologies affecting the intracranial segment may be variable. Depending on the location, extent and size of the occlusion in the artery, there may be mild symptoms or neurological sequelae, and even death can occur. (1, 2, 4, 7, 8, 10)

The vertebral artery is divided into 4 segments according to its course.

• ***Pars prevertebralis (Segment V1)***: This is the segment that extends from the point where the VA arises from the subclavian artery to the entrance in the transverse foramen. No branches come off from this segment.

• ***Pars transversaria (cervicalis) (Segment V2)***: This segment runs from the transverse foramen of C6 (generally) to the transverse foramen of C2. From this segment, the following branches emerge respectively:

- R. spinalis (gives off the r. radicularis and r. medullaris segmentalis branches)

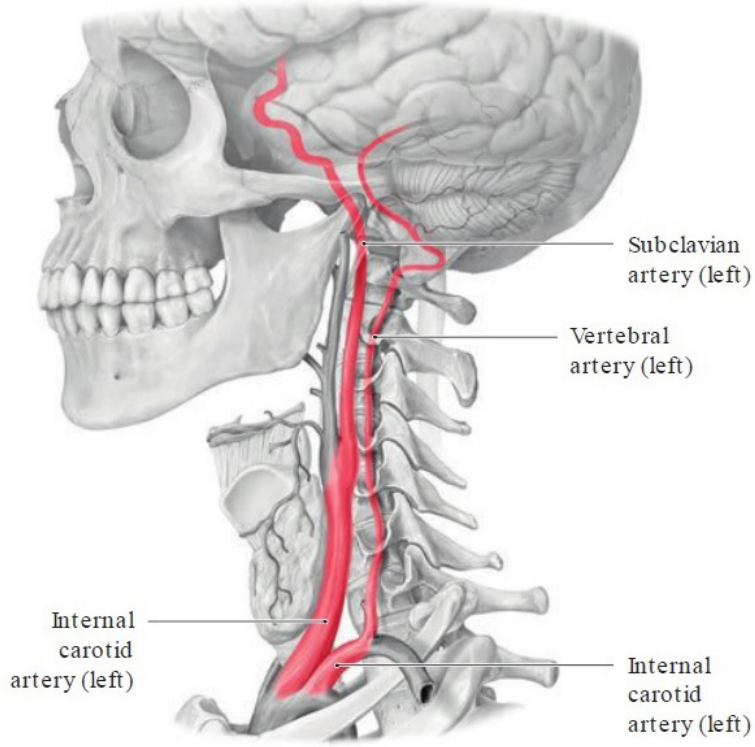
- R. muscularis.

• ***Pars atlantica (Segment V3)***: The segment that courses from the C2 vertebra to the foramen magnum. No branches arise from this segment either.

• ***Pars intracranialis (Segment V4)***: This is the segment that runs inside the cranial cavity. The branches originating from the artery during its course include:

- Anterior spinal artery
- Posterior spinal artery
- Rr. (Rami) meningei: At the level of the foramen magnum, they leave the VA and give off branches in the posterior cranial fossa between the periosteum and the bone. They supply the bone, periosteum and the falx cerebelli located in that region.
- Rr. medullares anteriores/ posteriores: Supply the medulla oblangata with its many small branches.
- Posterior inferior cerebellar artery (PICA) (1, 4, 7, 8) (Figure 2)

Figure 2. Left vertebral artery



Source: "THIEME Atlas of Anatomy". (9)

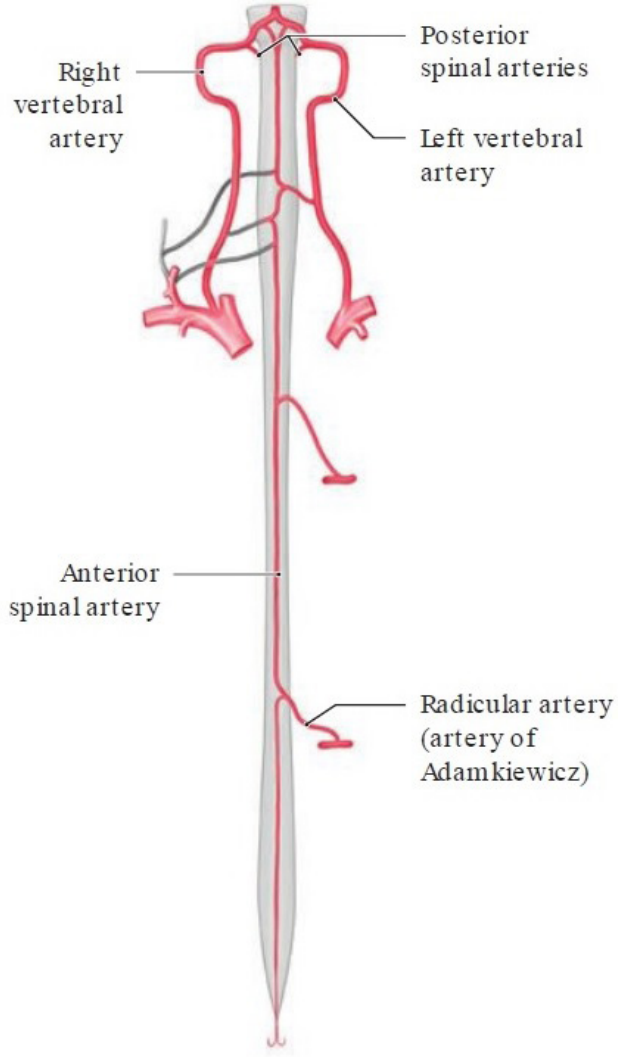
2.1.2. Rami spinales

Since the spinal cord (medulla spinalis) is a long structure, blood vessels from different segments supply this structure. While the cervical region is mainly supplied by the anterior and posterior spinal arteries, the thoracolumbar region receives blood through segmental arteries, which may vary from 25 to 30 pairs in number. About 12 to 14 pairs of these segmental arteries arise directly from the aorta, whereas the others originate from the VA, thyrocervical trunk, costocervical trunk and the internal iliac artery. The segmental vessels give off anterior and posterior branches immediately after emergence. The anterior branches (anterior intercostal arteries) supply the thoracic wall, while the posterior branches become the spinal artery and pass through the intervertebral foramina to enter the vertebral canal. The spinal artery pierces the spinal dura mater and splits into its anterior and posterior radicular branches. Among these vessels that supply the spinal ganglion and the nerve roots, anterior radicular branches do not reach the spinal cord. Some of the branches, ranging in number from 4 to 10, are broader than the others and contribute to the blood supply of the spinal cord by anastomosing with the anterior spinal artery. The posterior radicular branches enter the spinal cord through the radix posterior and anastomose with the posterior spinal artery. (2, 6, 8)

Another branch that goes to the spinal cord is the segmental medullary artery. During the development of the spinal cord, approximately 75% of the segmental medullary arteries regress and transform into anterior and posterior radicular arteries. The remaining segmental medullary arteries persist as such and reach the spinal cord via their radicular branches. (6)

On the left side, an artery emerging from the radicular arteries between the T8 and L3 segments is noticeably larger in diameter (in 90% of humans) than the other branches. This artery, which usually arises at the level of the lumbosacral intumescence (T9-T12), is known as the “arteria radicularis magna” (the large artery of Adamkiewicz). It supplies the adjacent lower thoracic, lumbar and sacral regions of the spinal cord. The blood supply to the area between the T4 and T8 segments of the spinal cord is poorer compared to the other segments. Therefore, paraparesis and paraplegia may develop due to a decrease in aortic blood pressure or during surgical interventions in that region. (2, 6, 7) (Figure 3)

Figure 3. Spinal cord arteries



Source: "THIEME Atlas of Anatomy". (9)

2.1.3. Anterior spinal artery

While the vertebral arteries extend upward and medially from the anterolateral surfaces of the medulla oblongata, two small branches branching off from the right and left vertebral arteries begin to descend and merge before exiting the foramen magnum. A single blood vessel is thus formed known as

the anterior spinal artery (ASA). On the anterior surface of the spinal cord, this artery runs along the midline, extending to the filum terminale. Within the filum terminale, the penetrating branches of the ASA form the sulcal vessels, which supply most of the gray matter and the anterolateral funiculus of the spinal cord. Approximately 75% of the blood supply of the spinal cord is provided by the anterior spinal artery. (1, 2, 7, 8) (Figure 4)

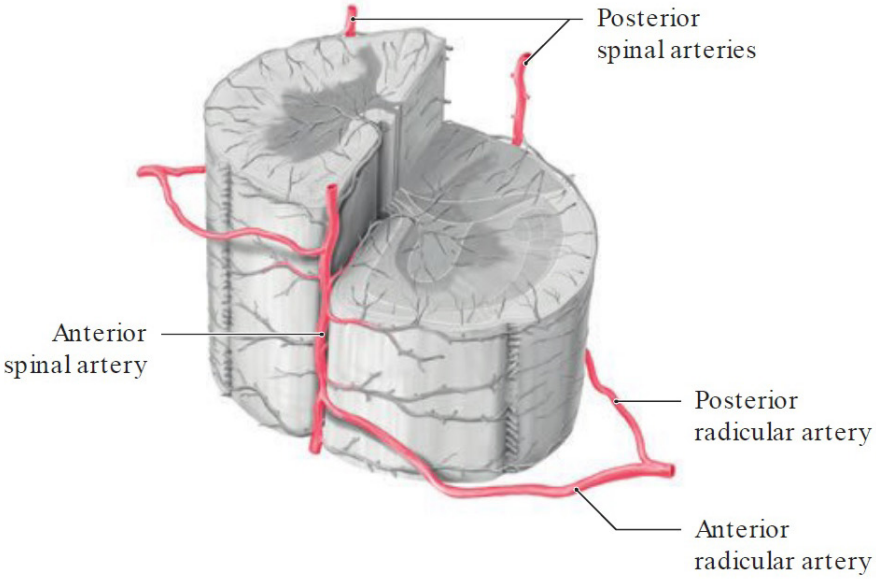
The proximal portion of the ASA supplies the pyramis bulbi, lemniscus medialis, fasciculus longitudinalis medialis, nucleus nervi hypoglossi and nucleus olivaris inferior. Blockage of the ASA results in a clinical condition known as the medial medullary syndrome. The symptoms of this syndrome include contralateral hemiparesis (except for the face), paresis of the ipsilateral tongue muscles and deep sensory loss depending on the loss of function in the area supplied by the ASA. Quadriparesis may occur in the case of bilateral involvement. (2, 7)

Blood flow to the ASA is about 1/1000 of the blood flow to the vertebral artery. In order to continuously meet the metabolic demands of the spinal cord, the anterior spinal artery forms many anastomoses with the posterior spinal artery and radicular arteries along its course in the vertebral canal. Thanks to these anastomoses, ischemic injury occurs less commonly in the spinal cord than in the brain. However, there are transition zones between these anastomoses, which create zones with low blood flow. In areas where collateral circulation is insufficient, ischemic injury may develop, usually in the upper-middle posterior region. (2, 7) (Figure 3)

2.1.4. Posterior spinal artery

The posterior spinal artery (PSA) arises as a pair from either the posterior inferior cerebellar artery or the vertebral arteries (in about 25% of humans). They descend in front of the dorsal roots of the spinal cord, supplying the posterior one-third of the spinal cord, including the posterior funiculus and posterior horn. Unlike the anterior spinal artery, the posterior spinal artery appears as a vascular network rather than a single straight blood vessel. The PSA forms numerous anastomoses with the anterior spinal artery at many levels on the surface of the spinal cord, and ends as a terminal artery after entering the spinal cord. (2, 4, 6) (Figure 4)

Figure 4. Anterior and posterior spinal arteries



Source: "THIEME Atlas of Anatomy". (9)

2.1.5. Posterior inferior cerebellar artery

The posterior inferior cerebellar artery (PICA) is the thickest branch of the vertebral artery. After leaving the intradural segment, the PICA passes behind the medulla oblongata and courses along the cranial roots of the glossopharyngeal, vagal and accessory nerves. It then travels through the anterior segment of the inferior cerebellar peduncle, winding around the cerebellar tonsil following a tortuous course to supply the posterior and inferior parts and nuclei of the cerebellum. When the PICA reaches the posterior lower part of the cerebellum, it bifurcates into two branches. These medial branches first supply the inferior vermis and then the choroid plexus after entering the lower half of the roof of the fourth ventricle. The lateral branches supply the cerebellar tonsil and the suboccipital cortical areas of the cerebral hemispheres. (1, 2, 7, 8) (Figure 1)

Immediately after leaving the vertebral artery, the PICA supplies the spinothalamic tract, posterior and anterior spinocerebellar tract, spinal nucleus of the trigeminal nerve, nucleus ambiguus and descending sympathetic tracts in the medulla oblongata. Thus, proximal occlusions of the PICA result in lateral medullary syndrome (Wallenberg syndrome). Affected individuals experience

severe dizziness, nausea, vomiting, nystagmus, ataxia, ipsilateral facial pain, pain in the contralateral arm, leg and trunk and loss of temperature sensation. Distal blockage of the PICA causes nausea, vomiting, balance problems and nystagmus. (2, 7)

2.2. Basilar artery

The basilar artery (BA) which is formed by the confluence of the left and right vertebral arteries in the bulbopontine sulcus may arise from the persistent primitive trigeminal artery independent of the vertebral arteries, albeit very rarely. On the anterior surface of the pons, it travels upwards in the pontine sulcus and terminates into the left and right posterior cerebral arteries at the pontocrural groove. From the basilar artery, several branches emerge including the anterior inferior cerebellar artery, pontine arteries, labyrinthine artery, superior cerebellar artery and lastly, posterior cerebral artery. (1, 2, 6, 8, 11, 12) (Figure 1)

2.2.1. Anterior inferior cerebellar artery

The anterior inferior cerebellar artery (AICA) is a blood vessel that exhibits considerable variations. Although the AICA emerges as a single branch from the lower segment of the basilar artery, it may sometimes give off two or three branches or may be totally absent. The AICA runs backward and downward from the pons, ventrally to the n. abducens, n. facialis and n. vestibulocochlearis and reaches the cerebellopontine angle. There, it branches off to supply the nerves entering from the internal acoustic meatus, and often winds to give rise to the labyrinthine artery. The AICA travels along the pedinculus cerebellaris medius and fissura cerebellopontinus to supply the base of the pons, the rostral bulb and the rostral cerebellar region (Figure 1). The AICA which supplies the inferior and anterior segments of the cerebellum anastomoses with the PICA and some of the branches of the superior cerebellar artery. For this reason, isolated AICA infarctions occur rarely. (1, 2, 6, 7)

2.2.2. Pontine arteries

Many thin branches emerging from both sides of the basilar artery, which are known as the pontine arteries, supply the basis pontis (Figure 1). The corticospinal tract located in the basis pontis is most affected by the occlusion of the basilar artery. Occlusion of the BA produces motor weakness that is usually bilateral and asymmetrical. In addition, damage to the corticopontocerebellar

tract and corticobulbar tract and associated ataxia, impaired coordination of the lower extremities, dysarthria of the head-neck muscles, dysphagia and dysphonia may occur. If bilateral damage occurs, quadriplegia may develop. (1, 2, 6, 7)

2.2.3. Labyrinthine artery

The labyrinthine artery often arises from the AICA (in 75% of humans) and supplies the cochlea and vestibulum in the inner ear. It may emerge from the basilary artery (in about 15% of humans) or the vertebral artery (in 5% of humans). The artery enters from the internal acoustic meatus along with n. facialis and n. vestibulocochlearis and then bifurcates into two branches, namely the common cochlear artery and the anterior vestibular artery. The common cochlear artery gives off the posterior vestibular artery and vestibulocochlear artery branches which supply the respective regions. Since the branches of the labyrinthine artery are end arteries, there is no collateral circulation. Therefore, severe pathologies may occur in the case of their blockage. (1, 2, 6)

2.2.4. Superior cerebellar artery

This artery exhibits the least variations and originates near the distal segment of the basilar artery. While traveling in the cerebellomesencephalic fissure, it supplies the dorsolateral surface of the mesencephalon, superior cerebellar peduncle and the upper half of the fourth ventricle. From there, it winds around the brainstem, passing above the trochlear nerve, below the trigeminal nerve and just underneath the oculomotor nerve, lying close to these nerves. It extends towards the cerebellum along the free border of the tentorium. Its lateral branches supply the upper outer surfaces of the cerebellar hemispheres and deep cerebellar nuclei. Isolated infarction of the superior cerebellar artery is uncommon due to its anastomoses with the AICA and PICA. (1, 2, 6, 7)

2.2.5. Posterior cerebral artery

As a terminal branch of the basilar artery, the posterior cerebral artery (PCA) bifurcates at around the level of the interpeduncular cistern. It runs parallel to the superior cerebellar artery and the oculomotor nerve passes between these two arteries. The PCA joins the branches of the internal carotid artery via the posterior communicating artery and takes part in the formation of the Circle of Willis (cerebral arterial circle). (1, 2, 4, 6-8, 11)

The PCA is divided into 4 segments depending on its course.

- ***Pars precommunicans (P1 segment)*:** This segment extends from the basilar bifurcation to the posterior communicating artery. The central branches emerging from this segment are known as perforating arteries. **Posteromedial central arteries** supply the thalamus, subthalamus, globus pallidus and the lateral wall of the third ventricle. Posterior choroidal arteries give off branches that supply the medial aspect of the thalamus, the walls of the third ventricle, corpus geniculatum mediale, fossa interpedincularis, substantia perforata posterior, pedinculus cerebri and the posterior portion of the mesencephalon.
- ***Pars postcommunicans (P2 segment)*:** The segment that runs around the brainstem after the posterior communicating artery. Its branches travel into the tegmentum, substantia nigra, nucleus ruber, corpus mamillare, hippocampus and fornix. **Posterolateral central arteries** supply the epiphysis, thalamus, corpus geniculatum mediale and diencephalon.
- ***P3 segment*:** The segment that extends from the lateral aspect of the mesencephalon to the sulcus parietooccipitalis and sulcus calcarinus. **Posterior choroidal arteries** are formed with the participation of the posterior communicating artery. They supply the corpus geniculatum laterale, other parts of the thalamus and the choroid plexus of the third ventricle.
- ***P4 segment*:** The last segment that gives off the **r. calcarinus** and **r. parietooccipitalis** branches.

Cortical branches arising from the P3 and P4 segments supply the occipital lobe, the posterior and inferior parts of the parietal lobe, and the anterior and inferior portions of the temporal lobe. (1, 2, 4, 6-8, 11) (Figure 1)

Occlusion of the perforating arteries leads to symptoms that vary depending on the region affected by ischemia, and include motor weakness if the corticospinal tracts are involved, somatosensory impairment if the thalamic afferent pathways are involved, memory loss if the corpus mamillare and hippocampus are involved, and disorders of the sympathetic and parasympathetic systems if the autonomous centers located in the diencephalon are involved. (2, 7)

3. Carotid system

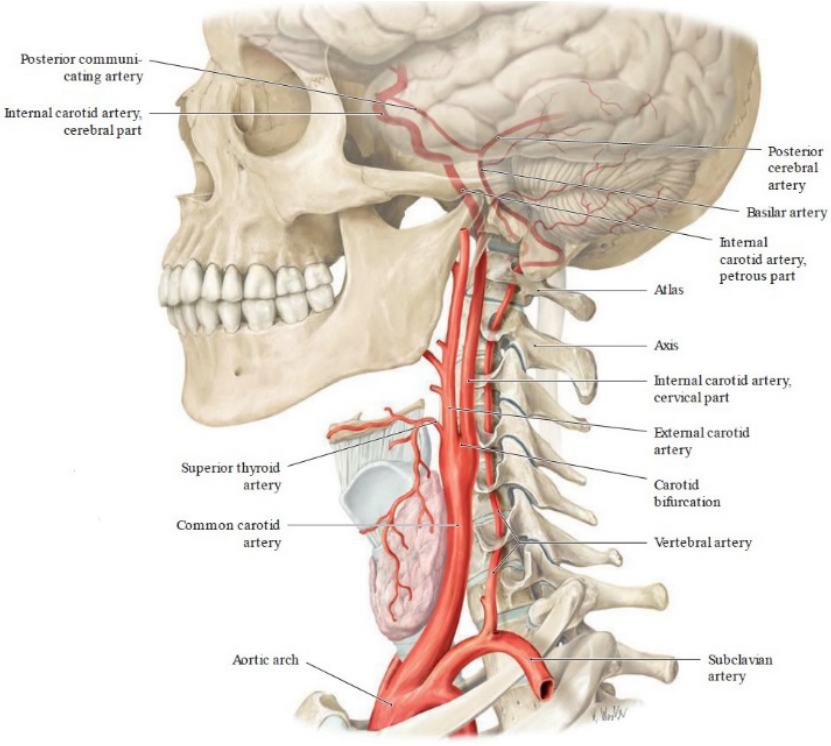
The common carotid artery, which emerges from the brachiocephalic trunk on the right and directly from the aortic arch on the left, gives off the internal carotid artery and external carotid artery branches at the level of the upper border

of the thyroid cartilage. The internal carotid artery is one of the main arteries that supply the brain. (1, 2, 4, 6-8, 11)

3.1 Internal carotid artery

After originating from the common carotid artery, the internal carotid artery (ICA) travels upwards laterally to the external carotid artery (in 50% of humans) without giving off any branches. As it ascends, it first passes posteriorly and then medially to the external carotid artery (ECA) and turns anteromedially within the carotid canal at a 90° angle. After exiting the carotid canal, it courses anteriorly within the cavernous sinus. Then it leaves the sinus at the level of the anterior clinoid process and enters the subarachnoid space. The ICA runs between the oculomotor and optic nerves and gives off the middle cerebral artery and anterior cerebral artery branches below the substantia perforata anterior and laterally from the chiasma opticum. (1, 2, 4, 6-8, 11) (Figure 5)

Figure 5. Left internal carotid artery



Source: "THIEME Atlas of Anatomy". (9)

The ICA is divided into four segments based on its course:

• ***Pars cervicalis*:** This is the segment of the artery before it enters the cranium. It travels within the vascular nerve bundle and extends to the base of the skull. No branches originate from this segment.

• ***Pars petrosa*:** The segment that travels within the carotid canal. There, it is separated from the middle ear cavity and the trigeminal ganglion by thin bony lamellae. In this segment, the ICA is surrounded by an extension of the dura mater and by the internal carotid plexus formed by the sympathetic fibers from the superior cervical ganglion. From this segment, the **a. caroticotympanic artery** and the **artery of the pterygoid canal** emerge. The caroticotympanic artery enters the tympanic cavity and anastomoses with other arteries present there. The artery of the pterygoid canal enters the pterygoid canal and anastomose with the branches of the greater palatine artery.

• ***Pars cavernosa*:** This is the segment that travels within the cavernous sinus. During its course in this area, it is laterally surrounded by the oculomotor, trochlear, maxillary, ophthalmic and abducens nerves. From this segment, the artery gives off the r. cavernosus, r. ganglionaris, r. meningeus and a. hypophysialis inferior branches.

- R. cavernosus: Supplies blood to the walls of the cavernous sinus and the nerves passing through it.

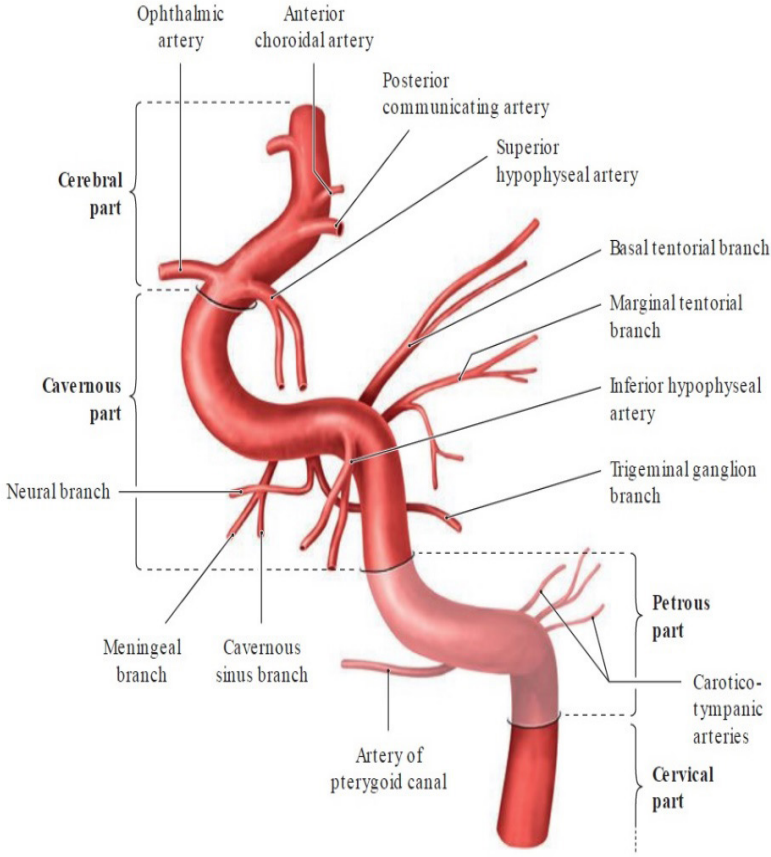
- R. ganglionaris: Gives branches to the trigeminal ganglion.

- R. meningeus: Supplies the dura mater of the anterior cranial fossa.

- A. hypophysialis inferior: Surrounds the infundibulum through anastomoses of its medial and lateral branches. The branches originating from this anastomotic network supply the neurohypophysis region.

• ***Pars cerebralis:*** This is the segment of the artery that extends from the point where it exits the cavernous sinus to its division to the middle cerebral artery and the posterior cerebral artery branches. The combination of the pars cavernosa and pars cerebralis is referred to as the “carotid siphon” due to its shape. From this segment, ophthalmic artery, anterior cerebral artery, medial cerebral artery, superior hypophyseal artery, posterior communicating artery and anterior choroidal artery branch off (Figure 6). (1, 2, 4, 6-8)

Figure 6. Segments of internal carotid artery



Source: "THIEME Atlas of Anatomy". (9)

3.1.1. Ophthalmic artery

The ophthalmic artery (OA) is the first branch of the internal carotid artery after it exits the carotid canal. It runs along the inferolateral aspect of the optic nerve and together they pass through the optic canal. The artery continues between the superior oblique muscle and the medial rectus muscle until it reaches the orbit. First it gives rise to its smallest branch, the central retinal artery. This branch travels within the optic nerve and supplies the retina. It is an end artery. The ophthalmic artery terminates in the dorsal nasal artery and the supratrochlear artery. The branches of the ophthalmic artery can be classified into two groups, including the orbital branches that supply the orbit and neighboring structures and the ocular branches that supply the eyeball and its muscles.

The orbital branches include:

A. lacrimalis: This is the thickest branch of the ophthalmic artery which supplies the lacrimal gland. One of its terminal branches, the lateral palpebral arteries anastomose with the medial palpebral arteries supply the conjunctiva and the eyelids . The r. meningeus recurrens branch of the lacrimal artery anastomoses with the medial meningeal artery, contributing to the blood supply of the dura mater.

A. supraorbitalis: This artery runs together with the n. supraorbitalis between the periosteum and the levator palpebrae superioris muscle until it reaches the supraorbital foramen. After passing through the foramen, it splits into its superficial and deep branches. The artery supplies the rectus superior and levator palpebrae superioris muscles and the forehead region.

A. ethmoidalis posterior: It passes through the foramen ethmoidale posterius and supplies the cellulae ethmoidales and the nasal cavity. One of its branches participate in supplying the dura mater.

A. ethmoidalis anterior: Together with the n. ethmoidalis anterior, it passes through the foramen ethmoidale anterius and gives off branches to the dura mater, cellulae ethmoidales anteriores and media, sinus frontalis and the nasal cavity.

Aa. palpebrales mediales: These arteries supply the eyelids.

A. supratrochlearis: Along with the n. supratrochlearis, this artery exits the orbit and reaches the forehead region. It anastomoses with the a. supraorbitalis and the contralateral a. supratrochlearis to supply the forehead area and frontal and ethmoidal paranasal sinuses.

A. dorsalis nasi: After giving off a branch to the lacrimal sac, it supplies the dorsum nasi by anastomosing with the a. angularis, r. lateralis naşi (a branch of the a.facialis) and the same branch from the opposite side.

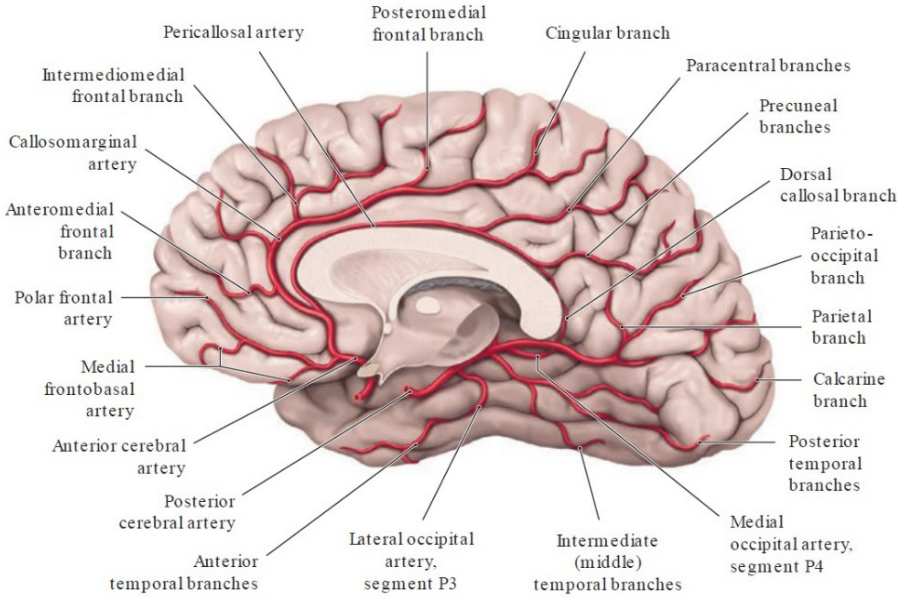
A. ciliares posterior longae, breves, anteriores: They travel around the optic nerve and pierce the sclera to supply the choroid layer of the eye and the ciliary process.

A. musculares: It supplies the rectus medialis, rectus lateralis, rectus inferior and obliquus inferior muscles. Some of its branches provide supply to the rectus superior, obliquus superior and levator palpebrae superioris muscles. (1, 2, 4, 6, 7, 11)

3.1.2. Anterior cerebral artery

After branching from the internal carotid artery at the medial end of the sulcus lateralis, the anterior cerebral artery runs anteriorly within the fissura longitudinalis cerebri. There, the middle cerebral arteries from both sides come very close to each other and connect with each other through a communicating artery known as the anterior communicating artery. Then, the artery runs backward within the sulcus corporis callosi. Its cortical branches supply the medial surface of the cerebral hemispheres until the parieto-occipital sulcus. The artery terminates in the pericallosal artery and callosomarginal artery branches. (1, 2, 4, 6-8, 11) (Figure 7)

Figure 7. Branches of the anterior and posterior cerebral arteries (medial view of the cerebrum)



Source: "THIEME Atlas of Anatomy". (9)

The segment of the anterior cerebral artery starting from its origin to the point where it gives off the anterior communicating branch is known as the A1 segment. The segment that continues backward over the corpus callosum is known as the A2 segment. The a. striata medialis proximalis and aa. perforantes

anteriores branches of the aa. centralis anteromedialis emerging from the A1 segment of the anterior cerebral artery and the aa. centrales anteromediales branches of the a. communicans anterior supply the deep structures of the brain including the internal capsule, thalamus, rostral parts of the basal ganglia, hypothalamus, optic chiasm and anterior commissure. The central branches supply the optic chiasm, lamina terminalis, area paraolfactoria, the medial part of the anterior commissure as well as the anterior regions of the hypothalamus. (1, 2, 4, 6-8) (Figure 1)

The branches emerging from the A2 segment of the anterior cerebral artery include a. striata medialis distalis, a. orbitofrontalis medialis, a. polaris frontalis and a. callosa marginalis (rr. frontales, r. cingularis, r. paracentralis, a. pericallosa, r. precuneus, r. parietooccipitalis). The first branch, a. orbitofrontalis medialis, supplies the olfactory bulb, gyrus rectus and the medial aspect of the gyrus orbitalis. The a. polaris frontalis, a. pericallosal and a. callosamarginalis supply the medial surfaces of the frontal and parietal lobes, as well as parts of the paracentral lobe, precuneus and gyrus cinguli. The a. striata medialis distalis (the recurrent artery of Heubner) which is longer than the other perforating branches supplies the striatum, genu anterior and crus anterior of the capsula interna, caput nuclei caudate and fasciculus uncinatus. (1, 2, 4, 6, 7) (Figure 7)

Blockage of the anterior cerebral artery produces symptoms that vary depending on the area affected by impaired blood supply. Since the medial surfaces of the cerebral hemispheres contain sensory and motor areas for the contralateral leg, the most common symptom is motor weakness in the proximal parts of the thigh and feet. If there is no involvement of the deep branches, no symptoms will occur in the face and hands. In the case of bilateral infarction, urinary and fecal incontinence occurs. Motor aphasia occurs if the anterior cerebral artery on the left side is affected. Involvement of the medial frontal lobe causes hyperactivity, emotional changes and anxiety. A pathological condition known as akinetic mutism develops if the anterior parts of the nucleus caudatus or gyrus cinguli are bilaterally affected. (2, 6, 7)

3.1.3. Middle cerebral artery

The middle cerebral artery (MCA) is the thickest branch of the internal carotid artery, and it runs laterally within the sulcus lateralis. Its branches supply the outer surfaces of the cerebral hemispheres and the insula, while its central branches participate in the blood supply to deeper structures. The branches of MCA are named according to the areas they supply. Anatomically, the MCA is

divided into two segments but surgically two additional segments are considered. (1, 2, 4, 6-8)

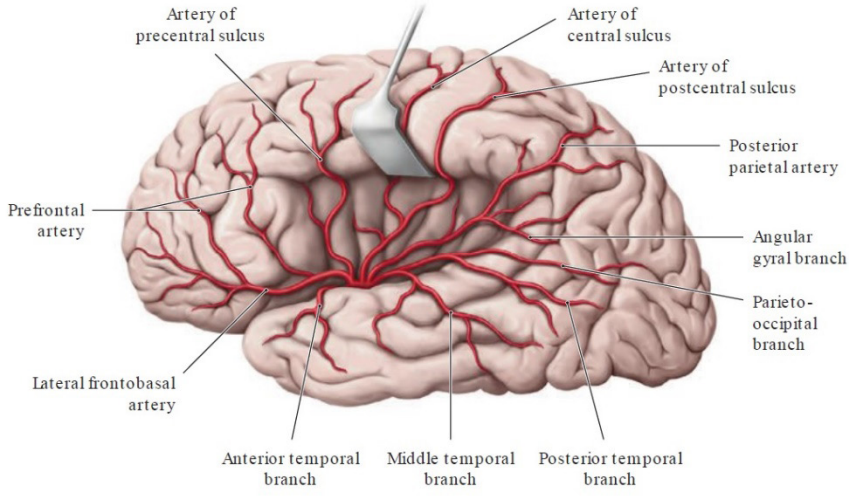
• ***M1 segment***: The segment that extends from the point where it exits from the internal carotid artery and bifurcates. Several branches emerge from the M1 segment including the aa. centrales antrolaterales, a. polaris temporalis and a. temporalis anterior-media-posterior. The central branches supply the region where basal ganglia are located including the internal capsule and the corona radiata.

• ***M2 segment***: This is the segment where the artery enters the sulcus lateralis and branches off over the insula. It only gives off the a. insularis branch and does not branch into the temporal lobe.

• ***M3 segment***: The segment where the middle cerebral artery gives off its opercular branches within the sulcus lateralis. Its branches include r. temporalis anterior-media-posterior, r. temporooccipitalis and r. gyri angularis.

• ***M4 segment***: The segment where the middle cerebral artery exits the sulcus lateralis and gives off cortical branches that supply the lateral surfaces of the cerebral hemispheres. Several branches originate from this segment including a. perforantalis, a. frontobasalis lateralis, a. sulci precentralis, a. sulci centralis, a. sulci postcentralis, and a. parietalis anterior and posterior. (2, 4) (Figure 8)

Figure 8. Branches of the middle cerebral artery (lateral view of the cerebrum)



Source: "THIEME Atlas of Anatomy". (9)

Ischemic stroke events most commonly involve the MCA and its branches. Due to the involvement of superficial cortical areas, motor weakness and sensory loss may occur. The symptoms vary depending on the affected area. If the gyrus precentralis and postcentralis are affected, contralateral hemiparesis and sensory loss are more likely to occur in the face and upper extremity. In the case of a lesion in the dominant hemisphere, Broca's aphasia may occur. If the lesion is in the temporal lobe and in the dominant hemisphere, Wernicke's aphasia may develop. If there is a blockage in the middle cerebral artery itself, contralateral hemiparesis occurs in the upper and lower extremities (2, 7)

3.1.4. Posterior communicating artery

The posterior communicating artery emerges from the internal carotid artery and then courses backward to join the posterior cerebral artery, completing the posterior half of the Circle of Willis. This artery often exhibits variations such as asymmetry, hypoplasia or aplasia. Branches originating from the posterior communicating artery contribute to the blood supply of the hypothalamus, pituitary gland, thalamus and hippocampus. (1, 2, 7)

3.1.5. Anterior choroidal artery

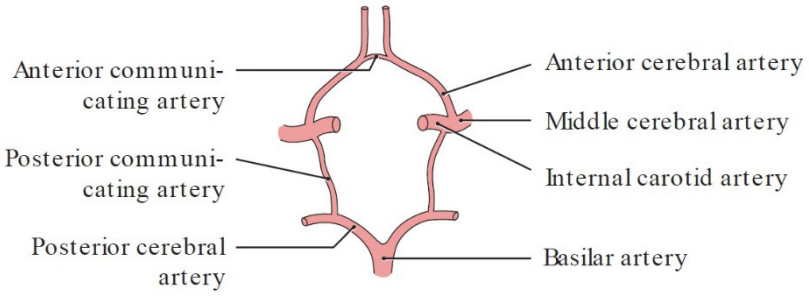
The anterior choroidal artery emerges from the internal carotid artery at the level of the optic chiasm. Then it extends posteriorly and terminates by anastomosing with the posterior choroidal artery in the inferior horn of the lateral ventricles. It supplies the choroid plexus, the globus pallidus, the crus posterius of the internal capsule the anterior parts of hippocampus, the uncus, the cerebral peduncle, rostral regions of the mesencephalon, the corpus geniculatum laterale and the posterior part of the radiatio optica. (1, 2, 6, 7)

Occlusion of this artery results in contralateral hemiplegia, sensory loss and hemianopsia, and ataxia in most of the cases. (2)

4. Circulus arteriosus cerebri (Willis polygon)

The Circle of Willis (Willis polygon) refers to the network of two main arteries (a. vertebralis and a. carotis interna) anastomose around the optic chiasm and the infundibulum of the pituitary gland. This polygonal arterial network is formed by anastomosis of the right and left anterior cerebral arteries connected by a single anterior communicating artery, the right and left posterior cerebral arteries (terminal branches of the basilar artery) and the posterior communicating artery originating from the internal carotid artery. This anastomosis protects the brain against ischemic strokes (Figure 9). (1, 2, 4, 6-8, 11, 13)

Figure 9. Circle of Willis



Source: "THIEME Atlas of Anatomy". (9)

The small branches (a. centralis cerebri) emerging from the arteries that form the Circle of Willis supply the brain's parenchymal tissue, and there are no anastomoses among them. These branches and the areas they supply are as follows:

A. centralis anteromedialis (a branch of a. cerebri anterior): It emerges from a. cerebri anterior and a. communicans anterior and enters the cerebral parenchyma via the substantia perforata anterior. It supplies the supraoptic and preoptic regions of the hypothalamus.

A. centralis anteromedialis (a branch of a. cerebri media): It also enters the cerebral parenchyma via the substantia perforata anterior and supplies the capsula interna and corpus striatum.

A. centralis posteromedialis (a. cerebri posterior): It emerges from the proximal part of a. cerebri posterior and supplies the pituitary gland, infundibulum, tuber cinereum, nuclei medialis thalami, subthalamus, and corpus mamillare. Its posteriorly extending branches supply the crus cerebri and the medial aspect of the tegmentum.

A. centralis posteromedialis (a.cerebri posterior): It originates after the point where the a. cerebri posterior anastomoses with a. communicans posterior, supplying the posterior part of the thalamus. (4)

The Circle of Willis is polygon-shaped in 45% of humans. Variations of the Willis polygon are commonly encountered and often (in 25-30% of the cases) they present as hypoplasia or aplasia of the posterior communicating artery, either unilaterally or bilaterally. Additionally, variations presenting as

embryonic vessels can be observed. These embryological remnants known as presegmental arteries are named as trigeminal, otic, hypoglossal or proatlantal based on their neighboring structures. They regress at embryonic weeks 5 to 8 but may persist into adulthood in about 1% of the general population. Among these presegmental arteries, persistent primitive trigeminal artery is the most commonly observed variation with an incidence of 85-87%, followed by the persistent hypoglossal artery, occurring in about 0.03–0.09% of the cases. Most of these variations are harmless and are incidentally detected during angiographic examinations. However, prior knowledge of these variations is essential to avoid complications during surgical procedures. (1, 2, 6, 12-17)

5. Cerebral veins (Venae cerebri)

The venous system of the brain, while not as clinically significant as the arteries, is important due to the unique characteristics of the cerebral veins. Knowledge of the course of the veins is crucial for neurosurgeons. Cerebral veins differ from other veins in our body in several aspects. Cerebral veins do not run parallel to the arteries, lack smooth muscle in their walls, do not have valves in their lumen, and after forming a network in the pia mater, they travel into the subarachnoid space and then drain into the dural sinuses. Unlike normal veins, the dural sinuses are composed of fibrous tissue. Lastly, the dural sinuses connect with each other and exit the cranium through the jugular foramen to drain into the internal jugular vein. (1, 2, 4, 6, 7, 11)

The cerebral veins are divided into two groups: venae superficiales cerebri and venae profundae cerebri.

5.1. Venae superficiales cerebri (Superficial veins of the brain)

They drain the cerebral cortex and the white matter of the brain.

Vv. superiores cerebri: They are responsible for the venous drainage of the upper, medial and lateral parts of the frontal, parietal and occipital lobes as well as the orbital surface of the frontal lobe into the sinus sagittalis superior. (1, 2, 4)

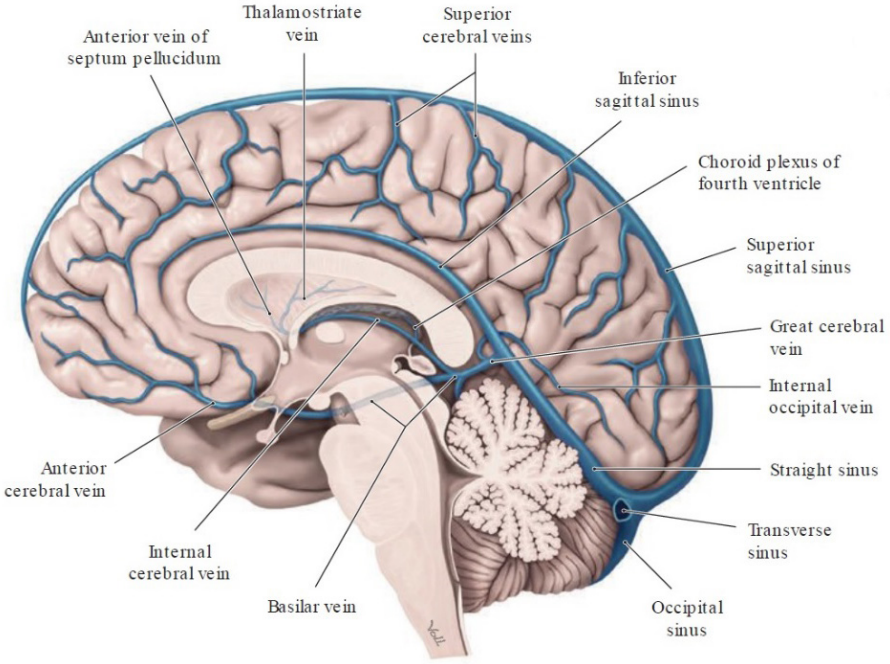
Vv. mediae superficiales cerebri: They drain the areas adjacent to the sulcus lateralis of the brain hemispheres and empty into the sinus sphenoparietalis and sinus cavernosus. (1, 2, 4)

Vv. inferiores cerebri: On the inferior aspect of the brain hemispheres, they drain the limbic lobe, corpus callosum, cortical parts of the rostral brainstem, gyrus parahippocampalis, paraterminalis, paraolfactorius, and uncus into the sinus sagittalis inferior, sinus petrosus superior, sinus transversus and sinus

cavernosus. However, the cortical veins show significant variability among individual making it difficult to define the boundaries of venous zones with sharp distinctions. (1, 2, 4, 6, 7, 11)

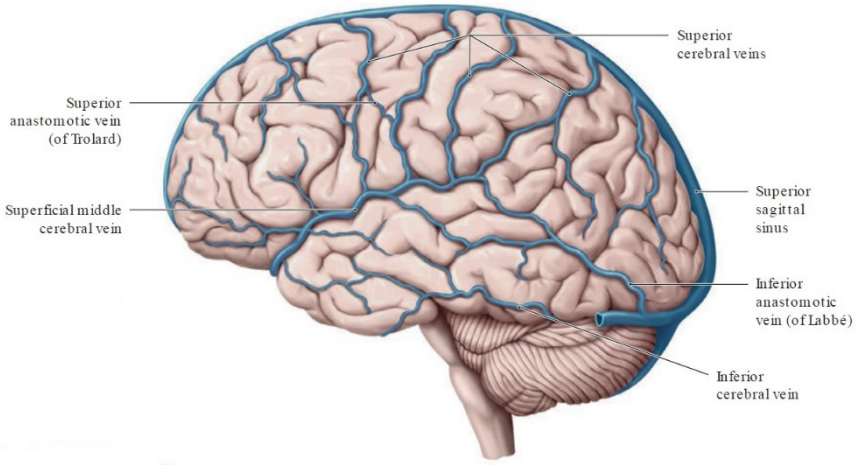
The superficial veins are connected through anastomotic veins. The superior anastomotic vein of Trolard is the broadest anastomotic vein and is located between the sinus sagittalis superior and the fissura cerebri lateralis. The inferior anastomotic vein of Labbe runs between the sinus transversus and the fissura cerebri lateralis. The superficial Sylvian vein either joins the sinus sphenoparietalis or drains directly into the sinus cavernosus. (2) (Figure 10,11)

Figure 10. Superficial veins of the brain (medial view of the cerebrum)



Source: "THIEME Atlas of Anatomy". (9)

Figure 11. Superficial veins of the brain (lateral view of the cerebrum)



Source: "THIEME Atlas of Anatomy". (9)

5.2. Venae profundae cerebri (Deep veins of the brain)

The deep veins of the brain collect venous blood from the basal ganglia located in the deep white matter of the brain, diencephalon, periventricular region and the choroid plexus. (1, 2, 4, 6)

Vv. internae cerebri: After collecting venous blood from the thalamus and the corpus striatum, v. thalamostriata merges with v. choroidea superior to form the v. interna cerebri. The right and left v. interna cerebri travel posteriorly and merge at the level of the cisterna quadrigeminalis to form v. magna cerebri (the great cerebral vein of Galen). (1, 2, 4)

V. basalis (the basal vein of Rosenthal): It is formed by the confluence of vv. anteriores cerebri, v. media profunda cerebri, v. gyri olfactorii and v. thalamostriatae inferiores. It collects the venous blood from the mesencephalon, hypothalamus, inferior horns of the lateral ventricles as well as draining v. choroidea inferior. (1, 2, 4)

V. magna cerebri: It is a vein formed by the confluence of the right and left internal cerebral veins with v. basalis, v. occipitalis and v. pericallosa posterior. It collects venous blood from the majority of deep cerebral veins and joins the sinus sagittalis inferior, ultimately emptying into the sinus rectus. (1, 2, 4)

The venous blood of the brain drains into the systemic circulation through dural venous sinuses. The venous blood coming from the cerebellum and the cerebral trunk drains into the neighboring sinuses. (1, 2, 4, 6, 7)

6. Conclusion

- The central nervous system is mainly supplied by 4 major arteries. Two internal carotid arteries and their branches supply most of the areas of the cerebral hemispheres except for the occipital lobe. Two vertebral arteries and their branches supply the remaining areas.
- The internal carotid artery terminates in a. cerebri media and a. cerebri anterior.
- A. cerebri anterior supplies the medial surfaces of the frontal and parietal lobes.
- A. cerebri media supplies the majority of the lateral aspects of the brain hemispheres.
- The two vertebral arteries join to form the basilar artery. The basilar artery gives off two terminal branches: the left and right posterior cerebral arteries.
- A. vertebralis and its branches supply the cerebral trunk, cerebellum and medulla spinalis.
- A. cerebri posterior supplies the thalamus, mesencephalon and the occipital lobe.
- The arterial network formed between the terminal branches of the basilar artery and the branches of the internal carotid artery is known as the circulus arteriosus cerebri (Willis polygon).
- The venous blood of the cortical and subcortical structures of the brain is collected by the superficial and deep cerebral veins. Eventually, they all drain into the dural sinuses. The dural venous sinuses connect with each other and finally exit the skull via the foramen magnum, draining into the internal jugular vein.

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CHAPTER II

NUCLEIC ACIDS

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The ability to reproduce is one of the most fundamental characteristics of living organisms. In all organisms, the genetic information is inherited from parents and passed on to offspring. Chromosomes are made of nucleic acids and proteins, and they facilitate inheritance. All cells contain macromolecules called nucleic acids, which are essential for the expression and storage of genetic material. There are two types of nucleic acids: Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are composed of subunits called nucleotides. They play different responsibilities within the cell. RNA is involved in protein synthesis, whereas DNA is in charge of the storage, expression, and transmission of genetic information. (1)

1. Nucleic acid components

The two nucleic acids, DNA and RNA, are biopolymers that are made of monomers called nucleotides.

1.1. Nucleotides and Nucleosides

The constituent parts of nucleic acids are called nucleotides. They are made up of phosphate, a pentose sugar, and a nitrogenous base. Nucleosides lack the phosphate group, which differentiates them from nucleotides. (2) Nucleotides are formed by the addition of a phosphoric acid moiety to the 5th carbon atom of the nucleoside. (3)

Nucleosides are formed by attachment of a purine or pyrimidine base to the 1st carbon atom of a 5-carbon sugar molecule. Depending on whether they are bonded with ribose or deoxyribose sugar, they can be classified as ribonucleosides or deoxyribonucleosides. (3)

1.2. Bases

Purine and pyrimidine bases joined with phosphorylated sugars make up the nucleotides. DNA and RNA are nucleotide polymers. There are two types of bases: purines and pyrimidines.

1.2.1. Purine bases

Purine bases have a double-ring structure consisting of nitrogen and carbon atoms. In essence, purines are pyrimidines fused with a second ring. This second ring is formed by the addition of two nitrogen atoms and one carbon atom to the single ring, which is the same as in the pyrimidine skeleton. Adenine and guanine are the large, double-ringed purine bases found in the structure of DNA and RNA. (3, 4)

1.2.2. Pyrimidine bases

Pyrimidine bases are small, single-ringed structures. They have a skeleton consisting of four C and two N atoms arranged in a ring. Cytosine, Thymine, and Uracil are pyrimidine bases. Cytosine is found in both DNA and RNA, while thymine is present only in DNA, and uracil only in RNA. (3, 4)

1.3. Sugars and Phosphoric Acid

Nucleic acids contain two types of pentose sugars known as deoxyribose and ribose. Deoxyribose is found in deoxyribonucleic acid (DNA), while ribose is present in ribonucleic acid (RNA). Deoxyribose sugar has a hydrogen (H) atom at the C-2' position, whereas ribose sugar has a hydroxyl (OH) group attached to the C-2' position. (3,5)

Both DNA and RNA contain phosphoric acid, and a nucleotide is formed by the addition of a phosphoric acid moiety to the 5th carbon atom of the nucleoside structure. Subsequently, nucleotides join together to form nucleic acids: ribonucleic acid and deoxyribonucleic acid (3).

2. Deoxyribonucleic Acid (DNA)

DNA was first isolated by Friedrich Miescher in 1869 who observed that the cell nucleus contained a substance he called "nuclein." Miescher, along with Albrecht Kossel and Richard Altmann, characterized "nuclein," and subsequently, nuclein was renamed to "nucleic acid" by Altmann. Then, it was demonstrated by Kossel that nucleic acids contain purine and pyrimidine bases, sugar, and phosphate. Later on, Erwin Chargaff showed that in the DNA

molecule of any species, the ratio of cytosine is always equal to that of guanine, and the ratio of adenine is always equal to that of thymine. (6) This discovery was crucial in understanding DNA base pairing. The "transformation principle" in bacteria was the subject of an article written by Oswald Avery, Colin MacLeod, and Maclyn McCarty in 1944. This article was the first to identify DNA as the genetic material. Between 1949 and 1953, Erwin Chargaff and his coworkers measured the amounts of the four bases in DNA samples of a variety of organisms. This important data played a key role in the development of the DNA model proposed by Watson and Crick. (5)

In 1938, William Astbury obtained the first X-ray diffraction pattern of DNA and showed that there were some elements in the DNA structure that repeated at intervals of 3.4 Å. Then, between 1950 and 1953, Rosalind Franklin, through her work with purer DNA samples and advanced X-ray data, confirmed the presence of these repeating structures at 3.4- Å intervals. Based on this finding, Franklin suggested that DNA has a helical structure. (5)

Almost all living organisms use DNA as the biomolecule for storing genetic information. DNA is composed of hydrogen, phosphorus, oxygen, carbon, and nitrogen atoms. (7) Nucleotides, which are monomer units, are joined to form the polymer DNA. A nitrogenous base, a 5′-carbon sugar known as deoxyribose, and one or more phosphate groups are all components of a nucleotide. The building blocks of DNA are nucleotides. Four distinct bases make up the DNA molecule, including single-ring pyrimidine bases (cytosine and thymine) and double-ring purine bases (adenine and guanine). The nitrogenous base is joined to the 1′-carbon of deoxyribose sugar and the phosphate group is linked to the 5′-carbon in each monomer. This bond is referred to as N-glycosidic bond. Because the phosphate group in DNA has an acidic nature, DNA is referred to as a nucleic acid. A phosphodiester bond connects the 3′-hydroxyl of one nucleotide in the DNA chain to the 5′-phosphate group of the next nucleotide. (6)

The sugar in DNA is deoxyribose, which has a ring structure containing one oxygen (O) atom and four carbon (C) atoms. A 5′ *carbon atom* is *attached* to the *4′ carbon* of the *ring*. In addition, d*eoxyribose* contains a *hydroxyl group* (*-OH*) *attached to the 3′ carbon of the ring*. Chromosomes are composed of two DNA polymers connected by non-covalent hydrogen bonds that make up a three-dimensional structure known as the double helix. (1)

James Watson and Francis Crick published their groundbreaking work on the established double-helix structure of DNA in the journal *Nature* in 1953.

Their research was built on data from various sources, including Chargaff's base composition data and Franklin's X-ray diffraction pattern of DNA, which played a significant role in helping them decipher the structure of DNA.

2.1. Watson- Crick model

In 1953, Watson and Crick announced their findings on the structure of DNA. The researchers proposed that DNA had a double-helix structure based on earlier discoveries. The characteristics of this model include:

1. DNA is made up of two polynucleotide strands that coil around a central axis to form a right-handed double helix.

2. 3'-OH and 5'-P ends of the two chains run in the opposite directions; hence, they are anti-parallel.

3. Sugar-phosphate backbone is located on the outer side of the molecule. On the inside of the double helix, the flat bases of both chains are piled on top of one another, 3.4 (0.34 nm) apart and perpendicular to the axis.

4.The formation of H bonds causes the nitrogenous bases of opposing chains to pair; in DNA, only A-T and C-G pairs occur.

5. The length of a complete turn of the helix is 10 base pairs; therefore, a full rotation of the helix is 34 Å (3.4 nm) long.

6. The major groove of the DNA double helix is broader and the minor groove is narrower. Along the length of the molecule, the major groove and minor groove wind in opposite directions.

7. The double helix has a 2.0 nm (20) diameter. (1, 5)

3. Ribonucleic Acid (RNA)

The existence of a messenger RNA (mRNA) that mediates the interaction between DNA and protein products was hypothesized by François Jacob and Jacques Monod in 1961. Later, it was discovered that the information needed for protein synthesis is transferred from DNA to the ribosome through mRNA. (1)

Ribonucleic acid (RNA) is composed of nucleotides consisting of nitrogenous bases and phosphate groups attached to ribose sugars, which are present in most living organisms and viruses. Adenine, guanine, uracil, and cytosine are the nitrogenous bases found in RNA. While RNA viruses can be double-stranded, RNA is usually single-stranded and can exist in various lengths and structures. The process of creating RNA from DNA is known as transcription, and the process of creating proteins from RNA is known as translation. (8) One

of the two primary groups of nucleic acids, RNA, is in charge of translating the genetic material found in DNA into proteins. Unlike DNA, RNA is typically single-stranded, and contains uracil in lieu of thymine, and ribose sugar instead of deoxyribose sugar in its structure. (5, 9)

Another difference between RNA and DNA is their ability to form branched structures using 2' hydroxyl groups. In 1954, George Gamow proposed the first code to determine the amino acid sequence directly from the DNA double helix. Francis Crick introduced the adapter theory, which postulated that a small RNA molecule might act as a messenger between nucleic acid units that code for proteins and amino acids.

Mahlon Hoagland and Paul Zamecnik later confirmed the existence of these adapters, which helped scientists comprehend the function of transfer RNA (tRNA) in protein synthesis. (10) RNA is an essential biological macromolecule found in all living cells that converts the genetic information stored in DNA into proteins. Various types of RNA are involved in the expression of genetic information, including mRNA, tRNA, and ribosomal RNA (rRNA). (9)

3.1. Structure and synthesis

The linear polymer RNA is made up of four distinct nucleotides, each of which contains the base adenine, cytosine, guanine, or uracil together with a 5-carbon sugar known as ribose and a phosphate group. RNA and DNA share a similar basic structure, with the exception that RNA contains uracil in place of thymine and ribose sugar in place of deoxyribose. RNA is typically short and single stranded. The nucleotide sequence of RNA is encoded in the genes found in DNA, and RNA polymerase enzymes copy this sequence from DNA. (9)

Proteins are created by RNA using amino acids. Twenty distinct kinds of amino acids combine to produce the basic building blocks of proteins. A ribosome begins decoding (i.e., translating) the mRNA codons as soon as it binds to an mRNA transcript and recruits tRNAs with the encoded amino acid. Conserved structural RNAs are involved in numerous cellular functions, including translation, RNA cleavage, protein localization, and gene regulation at both the transcriptional and translational levels. (11)

3.2. Types of RNA

Among the many different forms of RNA are mRNA, rRNA, tRNA, microRNA, small nuclear RNA, small nucleolar RNA, small RNA, small Cajal body-specific RNA, guide RNA, small interfering RNA, and transfer-messenger

RNA. The three RNA types that are most crucially engaged in protein synthesis among them are mRNA, rRNA, and tRNA.

Ribosomal RNA: All cellular proteins are synthesized in ribosomes, which are organelles. Ribosomes, which are necessary for protein synthesis, are formed by rRNA. The large and tiny ribosomal subunits make up ribosomes. The 30S small subunit and the 50S big subunit combine to form the 70S ribosome in prokaryotes. On the other hand, the 40S small subunit and the 60S big subunit combine to produce the 80S ribosome in eukaryotes. (8)

Messenger RNA: The genetic code required for the production of proteins is carried by mRNA, which is produced from DNA. Pre-mRNA, which has both coding and non-coding sections (exons), is created from a replicated RNA transcript in eukaryotes. Mature mRNA is created following the splicing process (maturation) of pre-mRNA (8).

Transfer RNA: tRNA carries amino acids to the mRNA located on the ribosomes A 3' acceptor site, 5' terminal phosphate, D arm, T arm, and anticodon loop make up the cloverleaf structure of tRNA. This is the type of RNA that transforms mRNA into proteins. With the aid of aminoacyl-tRNA synthetase, tRNA transports amino acids to a ribosome complex at its 3' acceptor site. (8)

microRNAs: MicroRNAs (miRNAs), which are short, highly conserved non-coding RNAs, regulate the expression of genes (12). In order to regulate post-transcriptional gene expression, miRNAs often silence or inhibit translation by the cleavage and destruction of the mRNA transcript. (13)

Small interfering RNAs: Small interfering RNAs (siRNAs) are non-coding, double-stranded RNAs that downregulate gene expression via RNA interference. They suppress gene expression by mRNA degradation and inhibition of protein translation (8). Using the RNA-induced silencing complex (RISC) as a mediator, siRNAs target and destroy gene transcripts. While siRNAs primarily attach to a single gene locus, miRNAs can control a wide range of gene targets through imperfect base pairing. (13)

Small nuclear RNA: Small nuclear RNAs are uridine-rich RNAs that are typically fewer than 300 nucleotides long. They are involved in the processing of RNA and can be found in the nucleoplasm (snRNAs) and nucleolus (snoRNAs) of eukaryotic cells. They form ribonucleoproteins (snRNPs and snoRNPs) by joining with specific proteins. (9)

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CHAPTER III

CURRENT TREATMENT APPROACHES IN CHRONIC AUTOIMMUNE ARTHRITIS DISEASES

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1. Introduction

Chronic autoimmune arthritis diseases occur when the body's immune cells see their own cells as enemies and cause pain and inflammation. When this inflammation is mostly in the joints, it is called chronic autoimmune arthritis diseases. Chronic autoimmune arthritis diseases are two diseases, rheumatoid arthritis and ankylosing spondylitis. (1)

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive damage and destruction of mobile joints with variable extra-articular findings. Joint damage begins early in the disease as a result of active inflammation and can lead to irreversible disability. In ankylosing spondylitis, however, the sacroiliac joint is mostly involved and causes pain in the hip and lower back. It has not been determined exactly what causes the diseases. It is thought that they mostly arise from genetic reasons, and it is known that smoking and low socioeconomic level have a share in addition to genetic factors. Diagnosis of RA is made by counting tender and swollen joints, laboratory tests, measurement of inflammatory activity, serological tests and joint films. The diagnosis of AS is made with back and low back pain, clinical measurements

and radiographic images because it causes inflammation in the sacroiliac joint. TNF-α and interleukins cause inflammation in RA and AS pathology. Therefore, current treatments and treatments under development target these molecules and are used for remission of the disease. Depending on the condition of the disease and the patient's tolerance, treatment with NSAIDs is started in the first step, but treatment is continued with methotrexate in patients who do not respond, or with immunosuppressive drugs and combinations in advanced disease states. (2)

In this study, current pharmacological treatment methods and drug molecules used in chronic autoimmune arthritis diseases were compiled.

2. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a complex and not yet fully understood disease that causes destruction of many different cell types, joints, often found in the small joints of the wrists, ankles, knees, elbows, and less frequently in the hips and shoulders. It is a heterogeneous disease with a pathophysiology. Loss of function and bone damage may occur in the joints affected by the disease. RA, a systemic inflammatory disease characterized by (3) polyarticular joint involvement, affects approximately 1% of the population and is 2 to 3 times more common in women than in men .

Although the exact cause of rheumatoid arthritis is not known, it is known that family history, that is, genetic factors, is of great importance. Also, consistently reported risk factors include smoking, low socioeconomic status, and education level. (4)

2.1. Incidence of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a disease seen all over the world and affects 1% of the world's population. Recent studies have revealed that there are 40/100,000 cases of RA annually worldwide and that women are more affected than men. The lifetime risk of RA in adults is 1 in 28, which is greater than 3% for women, and 1 in 59, which is about 2% for men. Female hormones have an important role in susceptibility to disease; some studies have shown that estrogen and prolactin stimulate the growth of B cell autoantibodies. (1)

2.2. Rheumatoid Arthritis Risk Factors

Factors that increase risk may include female gender, exposure to tobacco smoke, occupational dust (silica), air pollution, high sodium, red meat and iron consumption, obesity, low vitamin D intake and levels. (5)

2.3. Rheumatoid Arthritis Pathology

RA is complex and is thought to involve a number of different cells and molecules with many suitable targets for therapy. While the mechanisms of RA pathophysiology still remain to be defined, it is known that the synovial membrane forms the center of the immune-inflammatory process.

Damage to the joints in RA occurs when the pannus, which is formed by proliferation in the inner layer of the synovium lining the bone and joint space under the cartilage, and the pannus, which normally has weak cell and vascular structure as a result of proliferation , turns into a destructive tissue like a tumor tissue, progressing to the adjacent cartilage and bone and destroying it.

It is characterized by activation of resident synovial inflammatory cells, primarily macrophages, infiltration of lymphocytes and neutrophils in the synovium, and the production of an inflammatory environment that promotes proliferation of synoviocytes and fibroblasts and neoangiogenesis.

3. Ankylosing Spondylitis

Ankylosing spondylitis (AS), a common type of spondyloarthropathy, is a chronic inflammatory autoimmune disease that affects the spinal joints and causes severe, chronic pain; it can also cause spinal fusion in more advanced cases. Ankylosing spondylitis is the prototype disease within the spondyloarthropathies, a group of diseases mainly manifested by inflammation of the axial skeleton, peripheral arthritis and enthesitis (inflammation at the attachments of bone to tendons, ligaments and joint capsules). This disease is essentially a systemic disease and causes a large number of non-skeletal manifestations that have a significant impact on the patient's prognosis. These accompanying features include inflammatory bowel disease, acute anterior uveitis (iritis), and psoriasis. Ankylosing spondylitis most commonly begins when the patient is in their twenties, but the first symptoms usually appear in the late teens.

AS, an autoimmune disease, develops through complex interactions between genetic background and environmental factors. Although there has been some progress in recent years, the etiology of AS remains somewhat unclear. Studies to date have revealed some factors that may be involved in the occurrence of AS, including genetic background, immune reaction, microbial infection, and endocrinal abnormality. (6)

3.1. Incidence of Ankylosing Spondylitis

The prevalence of AS has a clear correlation with the positive rate of human leukocyte antigen (HLA)-B27 in certain populations. There is a strong association and familial clustering with the HLA-B27 antigen. AS is estimated to affect approximately 0.5% of the population, with a male/female ratio of roughly 2:1. (7) In contrast, rheumatoid arthritis is seen at a rate of about 1% in most populations. (8)

In the literature, it has been reported that men are responsible for the vast majority of AS cases, while the proportion of men and women in non-radiographic axial spondyloarthropathy is similar. (6)

3.2. Ankylosing Spondylitis Pathology

Immune cells and natural cytokines, particularly human leukocyte antigen (HLA) B27 and interleukin 23/17, are crucial in the pathogenesis of AS.

The pathogenesis of AS is very complex. Existing studies suggest that this may be the result of several complex mechanisms. Various hypotheses, such as the 'misfolding' hypothesis, attempt to elucidate the role of HLA-B27 in the pathogenesis of AS. Other non-HLA-B27 genes may influence the occurrence of ankylosing spondylitis through immune function and gene interaction. In addition, relevant studies have focused on the disorder in the IL-23/IL-17 axis and the abnormality in lymphocyte activation and differentiation. It has been reported that most of the immune cells and cytokines are involved in the pathogenesis of AS. In particular, the IL-23/IL-17 pathway plays a very important role in the development of the disease. Currently, the pathogenesis of AS is considered to involve mainly immune T cells, while B cells are also thought to be somewhat involved. There are some studies on the pathogenesis of AS mediated by B cells , and studies on this subject may be strengthened in the future. Although the pathogenesis of ankylosing spondylitis is not yet clear, current research results may have guided significance for clinical practice. (9)

4. Rheumatoid Arthritis Treatment

The goals in the treatment of patients with rheumatoid arthritis should be to reduce pain and inflammation, to improve quality of life and maintain joint function, to prevent disease progression and loss of joint function.

4.1. Non-Steroidal Anti- Inflammatory Drugs (NSAID)

NSAIDs are always used in rheumatology because of their effectiveness as anti-inflammatory and analgesic agents. NSAIDs exert their effects by inhibiting the enzymatic activity of COX enzymes. (10)

NSAIDs are all synthetic inhibitors of the COX active site, but subtle mechanical differences in the way NSAIDs interact and bind with the active site are responsible for some of the differences in their pharmacological properties. (11) Acetylsalicylic acid is the only covalent, irreversible modifier of COX-1 and COX-2, whereas other NSAIDs are all competitive inhibitors that compete with arachidonic acid for binding at the active site. In addition to their use in rheumatoid arthritis (RA) and osteoarthritis, NSAIDs are widely used in the symptomatic treatment of chronic musculoskeletal pain and other rheumatic diseases characterized by various forms of acute pain. NSAIDs vary widely in their chemical class, but they share the ability to block the production of prostaglandins. Most common NSAIDs inhibit both isoforms, although there are some differences in potency of COX-1 and COX-2. Despite improvement in pain and stiffness with NSAIDs, these agents generally do not reduce acute phase reactants or alter radiographic progression. They are used as the first line of treatment in rheumatoid arthritis. (12)

4.2. Glucocorticoids

Glucocorticosteroids are frequently used in the treatment of patients with rheumatoid arthritis. There is still insufficient data to support their effectiveness and safety. Glucocorticosteroids can be used systemically by different routes of administration, at different doses and for different durations. Some data suggest that different glucocorticosteroid regimens may delay the development of bone erosion in patients with rheumatoid arthritis. The toxicity of short-term therapy is relatively low. Bone mineral density of patients should be measured regularly in the treatment of RA. For long-term treatment, the development of osteoporosis is a serious problem. Concomitant treatment with calcitriol or bisphosphonates may reduce this risk. (13)

Corticosteroids can improve the symptoms of patients with rheumatic diseases. They may also have a disease-modifying effect in rheumatoid arthritis, but they are not first-line therapy. Side effects are related to dose and duration of treatment. Patients on long-term therapy should be informed about these and the lowest effective dose should be prescribed. Treatment should not be stopped

abruptly. Its basic mechanism of action involves passive diffusion of free circulating glucocorticosteroids across the plasma membrane and subsequent binding to specific intracellular cytoplasmic receptors found in all cell types. The activated hormone-receptor complex binds to specific sites within the DNA in the nucleus and causes modulation of specific genes. The favorable effects of glucocorticosteroids in patients with rheumatoid arthritis may be explained either by their general anti-inflammatory and immunomodulatory effects or by hypothetically more disease-specific mechanisms. (14)

Glucocorticosteroids are used as a single agent or in combination with other anti-rheumatic drugs. In short-term (duration of use <1 year) regimens, treatment is usually aimed at controlling symptoms during periods of high disease activity, either with unchanged anti-rheumatic therapy or until the efficacy of other newly initiated DMARDs becomes evident. This type of use is often called bridge therapy. Glucocorticosteroids are used for a long time in patients in whom other drugs cannot adequately control arthritic symptoms due to the occurrence of unsatisfactory effects or side effects. Glucocorticosteroids may also be prescribed to prevent progression of joint damage as assessed by radiological scores.

Two different treatment approaches can be mentioned in the treatment with glucocorticoids. The first is long-term treatment with low-dose oral glucocorticosteroids in addition to anti-rheumatic therapies, and the other is intensive early combination therapy, including short-term high-dose glucocorticosteroids. (15,16)

In general, side effects are more frequent and more severe at higher doses and longer treatment periods. Common side effects are peptic ulceration, moonshine, cataracts, osteoporosis, weight gain, acne, purpura, and skin atrophy. (17)

4.3. DMARD

All patients with RA are candidates for DMARD therapy. Although NSAIDs and glucocorticoids can relieve symptoms, joint damage can continue to occur and progress. DMARDs have the potential to reduce or prevent joint damage, preserve joint integrity and function, and ultimately reduce overall healthcare costs and maintain the economic productivity of the patient with RA .

Initiation of DMARD therapy should not be delayed for more than 3 months for any definitively diagnosed patient with persistent joint pain, significant morning stiffness or fatigue, active synovitis , persistent elevation in ESR or

CRP, or radiographic joint damage despite adequate treatment with NSAIDs. For any untreated patient with persistent synovitis and joint damage, DMARD therapy should be initiated promptly to prevent or slow further damage.

Disease-modifying antirheumatic drugs (DMARDs), including methotrexate (MTX), sulfasalazine, gold compounds, and antimalarials, have been proven to slow radiographic progression and RA mortality rates, with the exception of antimalarials. (18) The best use of DMARDs is during the first six months of illness, when the response to treatment is very high. (19) Methotrexate is the drug of choice in the treatment of RA and also reduces the side effects of biologic agents. The use of high-dose Methotrexate, combination therapy (Methotrexate, Sulfasalazine and Hydroxychloroquine) and fish oil as adjunctive therapy is rapidly increasing to better control the disease. (20) The use of other agents such as Azathioprine, Cyclophosphamide, Cyclosporine and D- penicillamine is limited due to safety concerns.

DMARDs commonly used in RA include Hydroxychloroquine (HCQ), Sulfasalazine (SSZ), Methotrexate (MTX), and Leflunomide. Less commonly used include Azathioprine (AZA), D- penicillamine, gold salts, Minocycline, and Cyclosporine. Many studies have demonstrated the benefit of DMARD therapy in RA. The results of these studies include control of signs and symptoms of joint involvement, changes in functional status and quality of life, and delayed radiographic evidence of erosion.

Many factors influence the choice of DMARD for each patient. Patients and their physicians should choose an initial DMARD based on its relative efficacy, ease of administration, monitoring program requirements, medication and monitoring costs (including physician visits and laboratory costs), time to expected benefit, and frequency and potential severity of adverse reactions. The physician should also consider patient factors such as the likelihood of compliance, comorbid diseases, the severity and prognosis of the patient's disease, and the physician's confidence in administering and monitoring the medication. Because of these many considerations, input from a rheumatologist is often an important component of the overall management plan when initiating DMARD therapy.

4.3.1. Sulfasalazine

Sulfasalazine is thought to result in suppression of NF-kB by directly inhibiting IkB kinases a and b. Its metabolite, sulfapyridine , is also responsible for some anti- arthritic effects.

SSZ can act more quickly than HCQ and can sometimes benefit 1 month after starting treatment. More importantly, the SSZ's It has been shown to delay the radiographic progression of RA. SSZ is generally well tolerated, and most side effects include nausea and abdominal discomfort that occurs during the first few months of treatment. The incidence of these side effects is reduced by starting with a low dose and then gradually increasing it. Leukopenia is an occasional, more serious side effect that can occur at any time and therefore periodic laboratory monitoring is required. The clinical response should occur within 4 months and the need for a change in therapy should be determined at that time. (21)

4.3.2. Hydroxychloroquine

Antimalarials are lipophilic weak bases and easily cross plasma membranes and accumulate in lysosomes, raising their pH from 4 to 6. Higher pH in lysosomes results in decreased chemotaxis, phagocytosis, and superoxide production by neutrophils. They inhibit the stimulation of Toll-like receptor 9 (TLR). TLRs are cellular receptors for microbial products that induce inflammatory responses through activation of the innate immune system. (1)

A number of studies have documented the symptomatic benefit of HCQ and SSZ over the last decade, particularly for patients with mild disease in the early stages. Although HCQ alone does not slow down radiological damage, early treatment with HCQ has a significant impact on long-term patient outcomes. Rash, abdominal cramps and diarrhea are rare side effects. HCQ is generally well tolerated and does not require routine laboratory monitoring, although patients may need periodic ophthalmologic examinations for early detection of reversible retinal toxicity. The risk of retinal toxicity increases when the dose exceeds 6 mg/kg. The duration of benefit can vary between 1 month and 6 months. (22)

4.3.3. Methotrexate

Many rheumatologists choose MTX as the first DMARD, especially for patients with more active RA. It has become the standard by which new DMARDs are evaluated because of its favorable efficacy, toxicity profile, low cost, and historical record in the treatment of RA. Randomized clinical trials, especially in patients with more severe disease, in RA It has demonstrated the effectiveness of MTX . (22) Longitudinal observational studies and randomized controlled trials suggest that MTX delays the progression of radiographic erosions. (21) Observational studies show that more than 50% of patients taking MTX continue the drug for more than 3 years, which is longer than other DMARDs. (23) RA

patients taking MTX are more likely to discontinue treatment because of side effects rather than lack of efficacy. (24) Stomatitis, nausea, diarrhea, and perhaps MTX- induced alopecia can be reduced without significant loss of efficacy with concomitant folic acid or folinic acid therapy. Relative contraindications for MTX therapy are pre-existing liver disease, kidney failure, significant lung disease, or alcohol abuse. As the most common adverse reaction to MTX is elevation of liver enzyme levels, liver function should be monitored, but the risk of liver toxicity is low. (23)

4.3.4. Leflunomide

They have revealed that Leflunomide can be used as an alternative to MTX as monotherapy, especially for patients who cannot tolerate or experience an inadequate response to MTX. (21) The reduction in RA disease activity and radiological progression rate with Leflunomide appears to be equivalent to a moderate dose of MTX. Leflunomide may also be useful as combination therapy with MTX in the absence of a complete clinical response with full dose MTX. Liver enzyme levels were elevated in 5% of patients receiving leflunomide and 60% of patients receiving MTX plus Leflunomide. Since enterohepatic circulation plays a major role in Leflunomide metabolism, Leflunomide has a long half-life. Obstructive biliary disease, liver disease, viral hepatitis, severe immunodeficiency, inadequate contraception and Rifampin therapy are contraindications to the use of Leflunomide. (1)

4.3.5. Cyclosporine

Cyclosporine is useful as monotherapy and has short-term efficacy similar to that of the D- Pen. However, the use of Cyclosporine has been limited by its toxicity, particularly hypertension and dose-related loss of renal function. The 20% loss in renal function with cyclosporine appears to be largely, if not completely reversible, upon discontinuation of the drug. Dose calculation is more critical with cyclosporine than with other DMARDs to prevent renal toxicity. Many drugs can increase cyclosporine levels and therefore increase the risk of nephrotoxicity. Therefore, Cyclosporine therapy is primarily limited to patients with refractory RA .

4.4. Biological Agents

Biologics have opened up new therapeutic horizons in RA but have also caused regulators and quality control to deal with many new problems and the high costs of healthcare providers.

Biological agents are designed drugs that target specific inflammatory cells, cellular interactions, and cytokines that mediate RA-related tissue damage. Such agents are designed to reduce the signs and symptoms of RA and slow the progression of the disease. (25)

Finding an effective targeted therapy has taken a long time. Despite many uncertainties, dramatic advances have occurred in the treatment of RA. Blockade of TNF-α and IL-6, inhibition of T-cell co -stimulation, and B-cell depletion are highly effective and currently available treatments for RA. While no head-to-head studies have compared these four approaches, effective treatments actually target a common ultimate pathway in the pathogenesis of RA. (26)

4.4.1. Anti-TNF Agents

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine involved in the pathogenesis of several immunological diseases, including rheumatoid arthritis (RA) and Crohn's disease (CD). In RA, a disease that affects an estimated 5 million people worldwide, TNF-α is the main mediator of joint damage caused by inflammation, which is the hallmark of this disease. A decrease in TNF-α level improves the signs and symptoms of RA and other diseases. Autoimmune diseases and the availability of TNF-α inhibitors have revolutionized the treatment of these diseases.

TNF-α is a proinflammatory cytokine with a broad spectrum of biological activity, primarily produced by activated monocytes and macrophages. (27) This cytokine causes vasodilation, increases vascular permeability and activates plaque cells, and is involved in the formation of fever, anemia, and cachexia. (28)

The nearly simultaneous registration of Etanercept and Infliximab for the treatment of RA in 1999 heralded a new era in the treatment of RA. Adalimumab was also registered a few years later, and two additional anti-TNF-α biologic agents, Certolizumab pegol and Golimumab are also approved in the US. The therapeutic efficacy of blocking TNF-α in the clinic was difficult to predict based on in vitro or in vivo data. The efficacy of anti-TNF-α agents has now been demonstrated in many controlled studies. In patients treated with methotrexate plus an anti-TNF-α agent, near-complete prevention of joint destruction was achieved at the group level. Subsequent studies have supported the use of anti-TNF-α agents in combination with Methotrexate in patients with early RA or in patients with established RA who have not yet been treated with Methotrexate.

Few studies have directly compared the efficacy of anti-TNF-α agents with other reasonable therapeutic options. In the study involving patients with proven RA who were not yet treated with Methotrexate, the Methotrexate plus Etanercept combination was superior to Methotrexate monotherapy both clinically and radiographically, whereas Etanercept as a monotherapy was clinically similar to Methotrexate and radiographically somewhere between the Methotrexate and Etanercept combination. In the Best study, immediate initiation of Methotrexate plus an anti-TNF-α agent proved better than initial monotherapy with Methotrexate alone. (2)

4.4.2. Infliximab

Binding of infliximab to TNF-α prevents TNF-α from binding to its receptors and blocks the initiation of intracellular signaling that leads to gene transcription and subsequent biological activity. Infliximab Its binding to membrane-bound TNF-α in vitro causes lysis of cell lines via a complement or antibody-dependent mechanism of cell cytotoxicity.

From the early stages of the disease, rheumatoid synovial inflammation is accompanied by a marked increase in angiogenesis. There is direct evidence of a reduction in the number of blood vessels in patients treated with Infliximab. The reduction in angiogenesis demonstrates the anti-inflammatory and antidestructive properties of Infliximab. (29)

Infliximab is effective in patients with rheumatoid arthritis, but the duration of the therapeutic effect is significantly reduced with each successive infusion of the drug. Therefore, the duration of effective treatment with infliximab may be limited but has not yet been determined. Anti- infliximab antibodies are frequently detected in patients receiving more than one Infliximab infusion. Acute infusion-related events were reported in up to 38% of patients receiving multiple infusions of Infliximab. In addition, the frequency of infusion-related events in patients with positive anti-infliximab antibody tests was more than 3 times higher than in patients with negative anti-Infliximab antibodies. (30)

4.4.3. Etanercept

Etanercept is a dimeric fusion protein consisting of 2 copies of the soluble, extracellular ligand binding domain of the human p75 receptor for TNF-α binding with the constant (Fc) portion of human IgG1. The drug is produced by recombinant DNA technology in a Chinese hamster ovary cell line. (31) One US approved indication for etanercept is rheumatoid arthritis.

Not all patients with active rheumatoid arthritis have a satisfactory response to DMARDs. Most patients do not achieve remission, many are dissatisfied with the response, and others become resistant to treatment after an initially satisfactory treatment experience. In addition, side effects lead to discontinuation of DMARDs in a significant proportion of patients. Combination regimens of 2 or 3 DMARDs have been studied and are more commonly used in patients with resistant rheumatoid arthritis. Specific anti-cytokine therapy is a new adjunct or alternative therapeutic strategy in patients with DMARD- resistant rheumatoid arthritis. Etanercept was the first specific anti-cytokine therapy approved for use in patients with rheumatoid arthritis.

Further studies are needed to better define the efficacy and tolerability profile of etanercept. In dose-finding studies with etanercept, no plateau in therapeutic efficacy has occurred. Therefore, the optimal dosage regimen remains to be defined. There is no evidence of tachyphylaxis after 6 months of treatment. It remains to be determined whether tolerance to the therapeutic effect of etanercept will improve with long-term treatment. It is also unclear whether etanercept can reduce the need for other antirheumatic drugs such as NSAIDs, corticosteroids and DMARDs . It is thought that this may lead to significant reductions in drug-related morbidity.

Etanercept is equally effective in patients regardless of whether they are receiving concomitant Methotrexate therapy, no tachyphylaxis, marked lack of neutralizing antibodies, and better tolerability profile with prolonged use of Etanercept This suggests that it may have significant advantages over Infliximab in rheumatoid arthritis. But there are no comparative trials between these two drugs. (32)

4.4.4. Adalimumab

Adalimumab is the first fully human monoclonal antibody to block TNF-α. Besides being administered subcutaneously, it has a longer half-life than Etanercept, allowing for a less frequent injection interval. The clinical efficacy of adalimumab in combination with Methotrexate has been previously demonstrated in patients with early aggressive RA and in patients unresponsive to other biological or non-biological DMARDs.

Biological response modifiers targeting TNF inhibition have become established in RA treatments in recent years. Adalimumab, Etanercept, and Infliximab have been shown to be consistently effective, significantly improving symptoms and limiting the progression of joint destruction and the risk of

disability in patients with RA in the later stages of the disease. The therapeutic efficacy of the three TNF-α antagonists is measured by their ability to bind soluble TNF and prevent its binding to the native TNF receptor. TNF blockade in combination with methotrexate significantly reduces clinical disease activity and arrests joint destruction in most patients with early or long-standing RA. Even if disease activity is not significantly reduced, TNF blockade has been shown to reduce joint destruction, suggesting that inflammation and joint destruction can be separated and independently controlled by TNF inhibition. These effects have been attributed to the role of TNF in the activation and differentiation of osteoclasts, which are mediators of bone resorption in both synovitis and RA.

Although TNF antagonists have made significant progress in the treatment of RA, some patients with RA may be intolerant or not achieve a clinically relevant response to these agents. It remains to be seen whether switching TNF blockers produces better clinical response and tolerability. There are studies showing that switching between the three available TNF antagonists (Adalimumab, Etanercept, and Infliximab) is safe and effective, with little error due to intolerance or lack of efficacy. New biologics for the treatment of RA, such as Rituximab and Abatacept, also influence the therapeutic decision faced by clinicians and patients. An additional TNF antagonist may be tried before switching to another biological agent, as current data show that failure to respond to one TNF antagonist does not prevent response to another. Additional data are needed to document the safety and efficacy of switching between TNF antagonists in clinical practice to guide clinicians in treatment selection after anti-TNF therapy has failed.

Adalimumab is effective regardless of previous TNF antagonist use. Adalimumab is well tolerated in patients intolerant of Etanercept and/or Infliximab. Adalimumab is also effective when used as a third TNF antagonist. (33)

4.4.5. Certolizumab Pegol

Certolizumab pegol is a pegylated, humanized anti-TNF Fab fragment. Because it lacks the Fc portion, it does not induce apoptosis through complement activation or antibody-dependent cell-mediated cytotoxicity. The pegylation process delays the elimination of this small antibody-derived protein, prolonging its half-life. Certolizumab is administered subcutaneously every other week and can be given as monotherapy or in combination with Methotrexate. (2)

It neutralizes membrane-associated and soluble human TNF-α, but, unlike İnfliximab and Adalimumab, does not contain an Fc domain, so it does not fix complement or cause antibody-dependent cell-mediated cytotoxicity in vitro.

Although TNF-α inhibitors have the same primary mode of action, patient responses to these therapeutics remain variable. The observed lack of response, loss of response over time, and tolerability issues suggest that additional treatment options may be needed.

4.5. Other Biological Agents

4.5.1. Abatacept

Abatacept binds to CD80/86, blocking the interaction of CD80/86 and CD28 on APC antigen presenting cells (e.g, macrophages, dendritic cells, B cells) and T cell, respectively. The co-stimulatory pathway leading to T-cell activation is inhibited. This results in normalized cytokine and autoantibody levels and inhibition of osteoclast activity. (34)

Abatacept is effective in patients who have not previously received MTX and who have had an inadequate response to non-biological DMARDs or TNF inhibitors. The efficacy of Abatacept is comparable to other biological agents. From a clinical point of view, the favorable safety profile of Abatacept, particularly with regard to serious infections, is of interest. However, rare side effects such as. Long-term data from patients are needed for effects on malignancies. Currently, there is insufficient data to support the use of Abatacept in monotherapy, so it has been suggested that Abatacept be used in combination with MTX or in combination with another conventional DMARD in case of MTX side effects or intolerance.

4.5.2. Anakinra

Anakinra, a recombinant human form of the interleukin-1 receptor antagonist (IL-1Ra), is approved for use in RA. IL-1, together with TNF, is a cytokine thought to play an important role in synovial inflammation and joint destruction in RA. IL-1Ra blocks the binding of IL-1 to the IL-1 receptor, thus preventing activation of target cells. (35) One study showed that combination therapy with Anakinra at a dose of 1 mg/kg or 2 mg/kg of MTX was more beneficial than treatment with MTX alone. (36)

4.5.3. Rituximab

Chimeric monoclonal antibody that targets the CD20 molecule expressed on the surface of B cells. It was first used to treat non-Hodgkin lymphoma and was later approved for the treatment of rheumatoid arthritis (RA) that has not

responded adequately to TNF inhibitors and DMARDs. Sustained efficacy in RA can be achieved with repeated courses of Rituximab. However, the optimal dose and retreatment schedule of Rituximab in RA have not yet been determined.

Rituximab is an effective and relatively safe biologic agent in the treatment of RA. Anti-TNF is a drug used in patients who are resistant or intolerant to biologics. Data on the long-term safety of Rituximab are needed. Infusion reactions appear to be a disadvantage of the drug compared to other available biologic agents, but their incidence decreases with glucocorticoid premedication and subsequent infusions. The effect of Rituximab-induced hypogammaglobulinemia on the long-term risk of serious infections and malignancies needs to be further investigated. (24,37)

4.5.4. Tocilizumab

Tocilizumab is a recombinant humanized anti-IL-6R monoclonal antibody that binds to both mIL-6R and sIL-6R, blocking the IL-6-mediated signaling pathway. (38)

IL-6 is produced by lymphoid and non-lymphoid cells such as T cells, B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, and various types of tumor cells. IL-6 has a broad spectrum of biological activities, including bone metabolism, inflammation, and cell growth and differentiation in a variety of target cells, as shown. IL-6, which is particularly relevant to RA, is involved in osteoclast activation and in fibroblastic takes part in synovial cell activation and contributes to pannus formation, joint destruction, and disintegration of bone and cartilage.

5. Ankylosing Spondylitis Treatment

The goal of AS treatment with pharmacological treatments is to improve and maintain spinal flexibility and normal posture, relieve symptoms, reduce functional limitations, and reduce complications. The basics of pharmacological treatment include NSAIDs and TNF-α inhibitors. Additional treatments include non-TNF inhibitor biologics Secukinumab, Methotrexate, and Sulfasalazine. In addition, the oral small molecule JAK inhibitors Tofacitinib and Filgotinib appear promising in clinical trials and are expected to receive approval for AS soon. Physical therapy, exercise, and abstinence from smoking are universally recommended for all AS patients, regardless of whether the disease is active or stable. (39) Compared with on-demand therapy, there is no clinical benefit from continuous NSAID therapy, whereas hypertension and depression are more common in people on continuous NSAID therapy. However, if symptoms reappear after NSAID drug discontinuation or dose reduction, continued

use is recommended. NSAID therapy is not selected based on the patient's history of NSAID administration, comorbidities, and risk factors for side effects. Good responses to NSAIDs include reduction in inflammatory back pain and functional improvement. Inadequate response to NSAID therapy is defined as active disease despite the use of at least two different NSAIDs at the maximum anti-inflammatory dose and duration. Analgesics, especially opioid-like drugs, may be used when NSAID therapy has failed or is contraindicated. TNFs are recommended for patients with high disease activity despite NSAID therapy. Biological agents should be used according to their indications and contraindications and patient comorbidities. (40) Also, treatment with Infliximab or Adalimumab is preferable to Etanercept treatment. (41) Predictors for a good response to TNFs are short disease duration, patient age ≤40 years, absence of enthesitis , and HLA positivity. Contraindications for TNFs include an active infection, tuberculosis, advanced heart failure, lupus, multiple sclerosis, and cancer. Sulfasalazine or Pamidronate is recommended instead of non-TNFi biologics such as Abatacept and tocilizumab in patients with active AS and contraindications for TNFs. If there is no significant improvement 3 months after the application, the treatment should be changed. If a 6-month trial does not result in clinical remission or reduction in disease severity, treatment should be changed. After unsuccessful treatment of the first TNFi, a second TNFi with less efficacy may also be effective. (42)

6. Conclusion

In AS, joint destruction reduces the patient's quality of life with many symptoms such as stiffness and pain in the joints, loss of function, bone damage, and additionally spinal infusion.

Although RA and AS mostly occur in individuals due to genetic reasons, it is thought that environmental factors may also trigger these diseases.

It is tried to detect the diseases in the early stages, to prevent their progression and to achieve remission. In addition to physical examination, joint films and the patient's history, high CRP and ESR values are helpful for diagnosis since RA and AS are manifested by inflammation in the body.

RA and AS treatments and the drugs used can be common. NSAIDs are given to patients who can tolerate it to help reduce pain and stiffness during exacerbations. It is aimed to stabilize the patient's condition by using pain relievers and muscle relaxants in combination with NSAIDs. DMARDs are preferred as second-line therapy in patients who cannot or cannot tolerate

NSAID treatment. While many DMARDs can be used in RA, the efficacy of only Sulfasalazine has been demonstrated in AS. If DMARD treatments are insufficient, TNF-α inhibitors developed against TNF-α, which plays a role in inflammation in the body, and immunosuppressive biological agents that suppress other inflammation pathways are decided and used by the physician according to the patient's condition.

Over time, new pathways and causes of disease are discovered in the pathogenesis of diseases. By comparing the effects of drugs targeting these pathways with methotrexate and TNF-α inhibitors, clinical trials are conducted for various drugs and the discovery of new molecules is being studied.

More research should be done on targeted drug molecules specific to chronic autoimmune arthritis diseases and studies should be conducted to ensure that they are included in the treatment protocol.

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CHAPTER IV

EXPERIMENTAL MYOCARDIAL INFARCTION MODELS AND CURRENT APPROACHES

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1. Introduction

Cardiovascular system diseases encompass a wide range; some affect the heart (myocarditis, coronary heart disease, hypertension, etc.), while others damage the arteries (atherosclerosis, etc.) or veins (thrombophlebitis, etc.). One of the most common cardiovascular diseases is myocardial infarction (MI), which is an acute disorder resulting from the complete or partial interruption of blood flow to the heart, leading to necrosis of the heart muscle tissue (myocardium) (1). This condition affects the integrity of the cardiovascular system and can lead to serious complications or the death of the patient. Such disruptions often occur primarily due to atherosclerosis of the coronary arteries. Atherosclerosis causes narrowing of the coronary arteries and damage to the walls of blood vessels, creating a foundation for the formation of blood clots and arterial blockages.

Cardiovascular diseases account for 30% of deaths in Europe and America, while they constitute 32% of global deaths (2). According to data from the Turkish Statistical Institute, approximately 505,000 people died in 2022. Cardiovascular diseases accounted for 34.5% of these deaths. When examined by underlying causes of death within the circulatory system diseases,

it was reported that 42.3% of the deceased died from ischemic heart diseases, 23.5% from other heart diseases, and 19.2% from cerebrovascular diseases (3). MI remains a leading cause of death and hospitalization due to cardiovascular diseases in Turkey and worldwide (3). Today, the incidence of MI is increasing even among individuals under the age of 40. Myocardial infarction is typically associated with conditions such as atherosclerosis, hypertension, and diabetes. In most cases, patients often have a painful form of MI, allowing doctors to diagnose the disease accurately and initiate treatment promptly (1).

Various factors can influence the outcomes of MI. According to the European Society of Cardiology, mortality due to MI depends on various risk factors with significant predictive power, such as age, gender, coexisting illnesses, and an elevated heart rate, as well as changes in specific laboratory findings (4,5). These factors are especially crucial in medical emergencies that occur in the early hours after the onset of symptoms. In such situations, early diagnosis can help control the condition, preventing or delaying half of the deaths, and even minimizing the risk of death.

The existence of a universal definition for myocardial infarction (MI), divided into various subtypes, has led to the global adoption of standardized diagnostic criteria. Nevertheless, in practice, this classification is not consistently implemented and is seen as overly intricate by some (6). This lack of consistency in diagnosis can have negative effects on patient outcomes in cases where MI diagnosis is uncertain. Furthermore, it has also compromised the reliability of MI diagnosis in clinical research. To address these challenges and to create awareness among healthcare providers that different mechanisms can lead to myocardial infarction with varying treatment outcomes, numerous studies have emerged proposing alternative clinical classifications. These novel classifications acknowledge that myocardial infarction can occur as a result of other underlying conditions or as a complication of cardiac procedures. If this approach aligns with clinical practice and gains international acceptance, it has the potential to reduce diagnostic uncertainty by offering more objective and specific criteria for diagnosis.

Presently, myocardial infarction is classified into five subtypes; however, there are contentious aspects within this classification. For example, Type 2 myocardial infarction currently encompasses both coronary-related mechanisms (such as spontaneous dissection, emboli, or vasospasm) and non-coronary mechanisms (due to factors like tachycardia, hypotension, hypoxia, or anemia). The latter can arise in underlying conditions, with or without coronary artery

disease, necessitating distinct treatments for each situation. This divergence in treatment approaches limits the practical utility of this diagnosis. Additionally, there is often uncertainty in distinguishing between Type 1 and Type 2 myocardial infarctions, as well as in differentiating Type 2 myocardial infarction from non-ischemic myocardial injury (7). Diagnoses of Type 4 and Type 5 myocardial infarctions are rarely employed to describe complications that occur during procedures. Furthermore, debates persist among cardiologists and cardiac surgeons regarding the threshold values for cardiac troponin elevation and the necessity for additional evidence to confirm the presence of a complication (8). In the following section, we delve into the primary factors contributing to the limited acceptance and uncertainty associated with these classifications.

Myocardial infarction can be recognized or pathologically defined through clinical features that include electrocardiographic (ECG) findings, elevation of biochemical markers (biomarkers) of myocardial necrosis, and imaging.

Recently, the fourth Universal Definition of Myocardial Infarction was announced simultaneously in The Journal of the American College of Cardiology (9), Circulation (10), European Heart Journal (11), and Global Heart (12), jointly sponsored by the American College of Cardiology. This definition is endorsed by cardiology associations. The fourth update to the Universal Definition of Myocardial Infarction has become necessary due to various reasons, such as the rise of high-sensitivity cardiac troponin (cTn) tests. While these tests have significantly altered the approach to evaluating myocardial infarction, there have been no changes in the clinical criteria for MI. Myocardial injury is still characterized by abnormal cardiac biomarkers in the context of acute myocardial ischemia. However, the enhanced sensitivity of cardiac troponin (cTn-T or cTn-I) assays has revealed multiple situations where myocardial injury can exist as a distinct condition, even when acute ischemic heart disease is not present.

In the past 40 years, our understanding of MI, including its causes, diagnosis, and treatment, has significantly advanced. In the early 20thcentury, MI was often considered a fatal event that could only be diagnosed through autopsy. Until the 1970s, it was conservatively treated with long bed rest and subsequently a sedentary lifestyle once clinical findings and accurate diagnosis were understood (13). However, since then, there has been a wealth of knowledge that has greatly changed our understanding of its pathogenesis and treatment options. The majority of these knowledge leaps have been driven by preclinical research.

In the early stages of drug development, safety and efficacy should be established in non-human organisms. *In vivo* rodent models of MI have played a vital role in the development of many drugs, including commonly used ACE inhibitors. Unfortunately, non-human models often cannot be successfully extrapolated to clinical practice because drugs can be ineffective or hazardous in humans in some cases. Additionally, those used as preclinical models for MI often fail to yield appropriate results as they do not sufficiently mirror the actual condition in humans. Therefore, in cardiovascular research aiming to mimic MI symptoms as closely as possible, multiple preclinical models are generally used together. The purpose of this section is to bring together myocardial infarction models developed to date, systematically presenting more comprehensible and comprehensive data.

2. In Vivo Experimental Myocardial Infarction Models

2.1. Ablation Models

Ablation injury techniques such as cryoablation, thermal ablation, and radiofrequency ablation offer fundamental advantages in providing solid and repeatable control over the size, shape, and location of the injury area. With these methods, it is possible to create a consistent size, shape, and transmural depth of injury in the target myocardial tissue. The size of the damaged area is independent of variations in coronary anatomy (14), making the resulting ablation scar more reproducible than injury caused by ligation. The location of the infarct can be controlled independently of coronary anatomy, and the transmurality of the infarct can be regulated (15,16).

As mentioned earlier, the most commonly used animal model to investigate myocardial remodeling associated with acute coronary occlusion (ACO) is the model of left anterior descending coronary artery (LAD) ligation. However, LAD ligation can lead to inconsistent infarct sizes, posing a challenge. Variations in surgical procedures and changes in the anatomical structure of LAD can result in different-sized infarct areas. Additionally, LAD ligation is associated with high mortality rates, necessitating a large number of animals for a study to be conducted successfully.

Recently, alternative models for ACO, including radiofrequency (17), thermal (18), or cryogenic injuries, have been developed (19). In the cryoinjury model, a pre-cooled metal probe with liquid nitrogen is used to create damage in cardiac tissue. One of the considerations in this model is the need to use the cooled probe multiple times to achieve a sufficient infarct size. While the probe has high

conductivity relative to tissue, its heat capacity is lower. Therefore, the probe rapidly heats up and makes tissue cooling (infarction) heterogeneous. Based on this method, a cryoinfarction model has been developed. The key feature of this model is the use of a portable liquid nitrogen delivery system. The model is highly reproducible, easy to apply, and can be established rapidly and reliably. It creates a consistent transmural infarct lesion independent of coronary anatomy and eventually leads to heart failure. This method is particularly suitable for evaluating innovative pharmacological and tissue engineering-based strategies and studying the remodeling process. The description of this model is based on the work by Curaj et al. (20).

2.1.1.1. Animal

The cryoinjury-induced myocardial infarction model is typically conducted using mice and rats. In the experimental application of this model, male mice weighing 25±5 grams and aged 2-3 months are commonly used as a reference.

2.1.1.2. Experimental Procedure

The cryoinjury-induced myocardial infarction model is performed in three stages: thoracotomy, pericardiectomy, and cryoinfarction. After creating a suitable environment, the mice are anesthetized with 3.5% isoflurane. The fur on the neck and chest area is shaved. Subsequently, the mouse is placed in a supine position on a hot plate or hot pad. Anesthesia is continued by covering the mouse's mouth and nose with a mask. The depth of anesthesia is assessed by pinching the hind limbs or tail with forceps to check reflexes. Subsequently, an intracutaneous injection of 0.03 mg/kg buprenorphine is administered for analgesia. After this step, the mouse's legs are extended and secured in an open position using tape or a stabilizer. The shaved area is disinfected with 10% povidone iodine, followed by two washes with 80% ethanol in the same area. A midline incision is made starting from the lower 1/3 of the sternum to the chin with scissors. Carefully separating the neck muscles surrounding the trachea with curved forceps, the trachea is exposed. A tracheotomy is performed between the 2nd and 3rd cartilage rings with a very small pair of scissors. Meanwhile, the ventilator frequency is set to 110/min, and the tidal volume is adjusted to 0.5 mL. After removing the face mask, the mouse is connected to the ventilator with a 20 G plastic cannula inserted into the trachea and ventilated. It is essential to ensure that both lungs are ventilated. Subsequently, the right pectoralis muscle is separated from the sternal attachments between the 3rd and 7th ribs using a

cautery. The 4th and 6th ribs are cut near the base of the sternum with an angled and spring-loaded scissors. It should be noted here to watch for bleeding from the mammary artery. If observed, the mammary artery is cauterized. Following the cutting procedure, the concentration of isoflurane is decreased to 2.5%. The connective tissue beneath is incised to achieve a clear view. Using blunt forceps, the pericardium is gently opened to expose the heart. A Goldstein retractor is employed to maintain the chest cavity in an open position. A blunt rod is utilized to lift the heart out of the chest cavity, while simultaneously reducing the tension on the retractor to prevent the heart from returning to its original position.

2.1.1.3. Creation of the Cryoinfarction Model

A cryoprobe with a diameter of 3 mm (9 mm in rats) is pre-cooled for 10 seconds for cryoinfarction. The probe is applied to the left ventricular anterior wall for 10 seconds (this can be repeated several times). After 10 seconds, cryo-damage resulting in an infarct occurs in the arteriolar part of the left ventricle. During this process, the probe adheres to the ventricular wall. Room-temperature saline is poured over the probe to separate it from the ventricular wall. This completes the cryoinfarction model. The application can be performed not only in the left ventricle but also in different regions of the heart as needed.

To reposition the heart, the chest opening is widened using a retractor, and the heart is gently placed back into position. The retractor is removed. Sternotomy is re-knotted using 6-0 sutures. The chest cavity is closed using continuous 6-0 sutures. Before tying the knot, any remaining air in the chest cavity is removed with a 10 cc syringe. The tissue is sutured from the caudal edge to the tracheal opening point with continuous 5-0 sutures. The isoflurane dose, which was at 2.5%, is reduced to 1.5%. The mouse is observed until it resumes spontaneous respiration. After spontaneous respiration, the catheter is removed. A mask is placed again for anesthesia to continue. The tracheal incision is closed with one 8-0 suture. The ventral neck muscles are restored to cover the trachea. The skin is sutured. To alleviate potential pain, metamizole is added to drinking water at a concentration of 50 mg/dL. The observation period for this model is up to 8 weeks.

The cryoinjury infarction model can be preferred for studying myocardial infarction and its complications. This model has a relatively low mortality rate. Additionally, rapid post-surgical recovery is observed. This model induces cardiac dysfunction, disturbances in the electrical conduction system, and transmural remodeling. Non-invasive evaluation can be performed *in vivo* in this model through electrocardiography and echocardiography.

2.1.2. Electrically Induced Myocardial Infarction

The use of rodent MI models is a fundamental basis for studies investigating MI processes and how they can be treated. The experimental MI model induced by electrical stimulation is performed with echocardiography support. The advantages of this model include being minimally invasive in mice and providing high repeatability. The disadvantages include requiring expertise in intervention and imaging, as well as the high cost of equipment.

This model is described with reference to the studies conducted by Sicklinger and colleagues (21).

2.1.2.1. Animals

In the electrically induced myocardial infarction model, male C57Bl/6 wild-type mice at 12 weeks of age with a weight of 25±2 grams were used

2.1.2.2. Experimental Procedure

1. Mice are anesthetized intraperitoneally with a dose of 100/90 mg/kg ketamine/xylazine. The depth of anesthesia is monitored by pinching the tail or hind legs. Eye cream is applied to prevent eye dryness.

2. The anesthetized mouse is placed in a supine position on the imaging platform. After this, the mouse's legs are placed in an open position and secured with a band or stabilizer. The ventral neck area of the mouse is shaved, and the shaved area is disinfected with 10% povidone-iodine. Subsequently, the same area is rinsed twice with 80% ethanol.

3. The LAD (left anterior descending coronary artery) is evaluated with high-frequency (HF) ultrasound while the mouse is under anesthesia.

4. In the B-mode imaging of anesthetized mice, the LAD is scanned from the root to the level of the middle papillary.

5. Color Doppler is used to visualize smaller branches.

6. A neutral electrode is attached to the mouse's right leg.

7. Then, a micromanipulator-controlled monopolar needle is slowly inserted into the closed chest cavity.

8. The needle is gradually directed towards the targeted area.

9. While the needle is on the LAD, HF electricity is coagulated using the coronary electrocoagulation unit. Note: The correct placement is confirmed by the loss of Doppler signal and akinesia in the affected area of the left ventricle.

10. Subsequently, the needle is slowly withdrawn.

11. The formation of myocardial infarction or occlusion, in other words, is confirmed by the absence of blood flow distal to the obstruction, akinesia in the affected part of the left ventricle, and typical EKG changes within seconds.

12. Cardiac morphological changes, cTn-T or cTn-I, and electrocardiographic and echocardiographic parameters are evaluated.

2.2. Minimally Invasive Surgery in the Model of Myocardial Infarction

In this model created by minimally invasive surgical intervention, application is performed without requiring intubation or tracheotomy. Instead of endotracheal intubation, a short cannula is used to maintain the animal's respiratory function. A thoracic incision is made in the intercostal area, minimizing damage to ribs and surrounding tissues. This results in a shorter recovery time and faster healing. This method can be used in both permanent ischemia and ischemia/reperfusion studies. The description of this model refers to the studies conducted by Curaj and colleagues (22).

2.2.1. Animals

The model study used male C57Bl/6 wild-type mice, 8-10 weeks old, weighing 25±2 grams.

2.2.2. Experimental Procedure

The mice were anesthetized intraperitoneally with a dose of 100/90 mg/kg ketamine/xylazine. The depth of anesthesia was monitored by pinching the tail or hind limbs. Eye ointment was applied to prevent drying of the eyes. In addition, 0.1 mg/kg of buprenorphine was used to reduce potential pain.

2.2.3. Surgical procedures

The procedures are as follows:

1. The mouse, under anesthesia, is placed in a supine position on a hot plate or hot pad. After this step, the mouse's legs are extended and secured in an open position using a band or stabilizer.

2. The ventral neck area of the mouse, specifically the left chest area, is shaved. After shaving, the shaved area is disinfected with 10% povidone-iodine.

3. Subsequently, the same area is swabbed with 80% ethanol twice for further sterilization.

4. A small incision of 0.5 cm is made in the middle of the neck. Using sterile curved forceps, the trachea is visualized by passing through the fat pads beneath the skin, and the trachea is viewed under a stereomicroscope.

5. An intubation cannula is orally placed into the trachea using this procedure. The cannula is then connected to a ventilator with a tidal volume of 0.1 mL and a frequency of 100 breaths per minute.

2.2.4. Creating the Myocardial Infarction

Here are the steps for creating the myocardial infarction:

1. Make a skin incision of less than 0.5 cm along a line between the xyphoid and the left axilla of the mouse.

2. Use forceps to separate the muscle layer. Separation is done between the ribs.

3. Create a small incision between the ribs until the thoracic cavity is opened (23).

4. Pay attention to whether the study is a chronic ligation model or an ischemia/reperfusion model. If it's a chronic ligation model, the incision should be made between the fifth intercostal space. If it's an ischemia/reperfusion model, the incision should be made from the fourth intercostal space.

5. To expose the heart, place retractors in the incision area, expanding the thoracic cavity.

6. Carefully remove the pericardium to minimize fibrotic processes.

7. The left anterior descending artery (LAD) will be visible as an open red vessel. For the chronic infarction model, place a ligature around the LAD, following the reference of the ligature positioning in the middle of the ventral side of the heart (between the atrium and the apex). Tie both branches of the artery with 7-0 silk sutures for transmural anterior and posterior infarctions.

8. For the ischemia/reperfusion model, place the ligature below the atrium, over the main stem of the LAD. Secure it with a loop on a silicone tube to preserve vascular integrity.

9. Place temporal sutures over the ribs during ischemia and use a compress to keep the tissue moist.

10. After the ischemic period, remove the silicone tube and cut the sutures, then observe perfusion.

11. If needed, provide a supplement of 0.5% isoflurane in addition to the used anesthetics and analgesics.

2.3. Chronic Coronary Artery Ligation

The chronic coronary artery ligation procedure, also known as permanent ligation, has been used in mice for over 60 years. Generally, the model has undergone very few changes, except for some technical improvements (24-26).

This model is commonly used to assess myocardial infarction (MI) and long-term cardiac remodeling following MI. It is often employed to model acute ST-elevation myocardial infarction (STEMI). In this model, the surgical occlusion of the coronary left anterior descending artery (LAD) results in a permanent blockage. This blockage leads to irreversible hypoxia in a significant portion of the left ventricle, causing distal perfusion to be impeded. This, in turn, leads to cardiomyocyte cell death, apoptosis, and the formation of infarct scar tissue.

The chronic coronary artery ligation model demands a high degree of proficiency in microsurgical techniques. Potential complications associated with this model encompass tissue damage and elevated perioperative mortality rates, primarily stemming from intraoperative bleeding, lung damage or collapse, ligation errors, or insufficient ventilation (27). One significant advantage of using the permanent ligation method is that it results in more pronounced differences in heart function between induced extensive infarctions and sham or MI animals. However, it's important to note that the permanent occlusion and the resulting large infarctions are less commonly observed in humans.

References for describing this model include studies by Curaj et al. and Martin et al. (20,28).

2.3.1. Animals

The chronic coronary artery ligation model is generally used in both mice and rats. In the experimental application of this model, male mice that are 2-3 months old and weigh approximately 25±5 grams are commonly used as a reference.

2.3.2. Experimental Procedure

In the chronic coronary artery ligation model, the procedure is performed in three stages: thoracotomy, pericardiotomy, and cryoinfarction. Once an appropriate environment is established, the mice are anesthetized with 3.5% isoflurane. The fur on the neck and chest areas is shaved, and then the mouse is placed in a supine position on a hot plate or hot pad. Anesthesia is continued by

covering the mouse's mouth and nose with a mask, and the depth of anesthesia is monitored by pinching the hind legs or tail to check reflexes.

Next, an intraperitoneal injection of 0.03 mg/kg buprenorphine is administered for analgesia. Following this, the mouse's legs are placed in an open position and secured with a band or stabilizer. The shaved area is disinfected with 10% povidone iodine and then washed with 80% ethanol twice.

A small incision, less than 0.5 cm, is made in the middle of a line between the xyphoid and the left axilla. Forceps are used to separate the muscle layer. A small cut is made between the ribs until the chest cavity is opened. It is important to note whether the study is a chronic ligation model or an ischemia/reperfusion model. For the chronic ligation model, the incision is made between the fifth intercostal spaces, while for the ischemia/reperfusion model, the incision is made between the fourth intercostal spaces.

To expose the heart, retractors are placed in the incision area, and the thoracic cavity is expanded. Careful removal of the pericardium is performed to minimize fibrotic processes. The LAD coronary artery will be visible as a prominent red vessel. In the chronic infarction model, a ligature is carefully positioned around the LAD, specifically targeting the midsection of the heart's ventral side, situated among the atrium and the apex. This ligature is applied using 7-0 silk sutures to induce transmural anterior and posterior infarction by ligating both branches of the artery.

For the ischemia/reperfusion model, the ligature is placed under the atrium, above the main stem of the LAD, and secured with a tie around a silicone tube to maintain vascular integrity. Temporal sutures are placed over the ribs during ischemia, and a compress is used to keep the tissue moist.

After the ischemic period, the silicone tube is removed, and sutures are cut to observe reperfusion. In addition to the anesthesia and analgesics used, supplemental 0.5% isoflurane may be administered to the mouse.

The procedure for returning the heart to its place involves expanding the chest opening using retractors with the help of an ekartor. The heart is gently placed back in its position, and the ekartor is removed. Sternotomy is resealed with 6-0 sutures. The chest cavity is closed using continuous 6-0 sutures. Before tying the knot, any remaining air in the chest cavity is removed using a 10 cc syringe. Tissue is sutured continuously with 5-0 sutures from the caudal edge to the tracheal opening. The isoflurane dosage is reduced from 2.5% to 1.5%. The mouse is observed until it resumes spontaneous respiration.

After spontaneous respiration, the catheter is removed, and a mask is used to continue anesthesia. The tracheal incision is closed with an 8-0 suture. Ventral neck muscles are restored to cover the trachea, and the skin is sutured. To alleviate potential pain, metamizole is added to drinking water at a concentration of 50 mg/dL. The observation period for this model is up to 8 weeks.

2.4. Ischemia/reperfusion Model

Inducing myocardial infarction (MI) in rodents through ischemia/reperfusion (IR) is a relatively recently developed model. The model procedure was initially used in ex vivo organs and was first established in dogs in 1988 (29,30). The procedure closely resembles the permanent ligation model, with the distinction that the occlusion is created by suturing a small piece of silicone tubing onto the Left Anterior Descending coronary artery (LAD), and after a temporary period, this suture is cut. Upon cutting the suture, the tube is removed, and reperfusion is reinstated. The literature reports variable occlusion durations ranging from 15 minutes to 120 minutes, with the most commonly used duration typically being 30 minutes (27,31-33).

References for describing this model include studies conducted by Curaj et al., Martin et al., and De Villiers et al. (20,28,29).

2.4.1. Animals

In the ischemia/reperfusion model, mice and rats are commonly used. In the experimental application of this model, male mice weighing 25±5 grams, aged 2-3 months, were used as a reference.

2.4.2. Experimental Procedure

In the ischemia/reperfusion model, which consists of three stages: thoracotomy, pericardiectomy, and cryo-infarction, the procedure is performed as follows:

1. After creating an appropriate environment, the mice are anesthetized with 3.5% isoflurane.
2. The fur on the neck and chest area is shaved.
3. The anesthetized mouse is placed in a supine position on a hot plate or hot pad. Anesthesia is continued by covering the mouse’s mouth and nose with a mask.
4. The depth of anesthesia is monitored by pinching the hind legs or tail to check for reflexes.

5. Intracutaneous injection of 0.03 mg/kg buprenorphine is administered for analgesia.

6. The mouse's legs are placed in an open position and secured with a band or stabilizer.

7. The shaved area is disinfected with 10% povidone-iodine.

8. The same area is then scrubbed twice with 80% ethanol.

9. Using scissors, make a small incision starting from the lower 1/3 of the sternum and extending to the chin along the midline.

10. Carefully separate the neck muscles that surround the trachea using curved forceps, exposing the trachea.

11. Create a tracheostomy between the 2nd and 3rd cartilage rings using a very small pair of scissors.

12. Adjust the ventilator to a frequency of 110/min breaths and a 0.5 mL tidal volume. Connect a 20 G plastic cannula placed in the trachea to the ventilator. Ensure that both lungs are ventilated.

13. After removing the facial mask, secure the mouse to the ventilator.

14. Using a cautery, separate the right pectoralis muscle from the sternal attachments between the 3rd and 7th ribs.

15. Use an angled and spring-loaded scissors to cut the 4th and 6th ribs just at the base of the sternum. Be cautious of any bleeding from the mammary artery; if observed, cauterize the mammary artery.

16. Lower the isoflurane concentration to 2.5% for better visibility.

17. Cut the underlying connective tissue to obtain a clear view.

18. Open the pericardium with blunt forceps to expose the heart. Use a Goldstein retractor to keep the chest cavity open.

19. The LAD artery can be seen as a bright red vessel. For transmural anterior and posterior infarctions, ligate both branches of the artery using 7-0 silk sutures.

20. For the ischemia/reperfusion model, place the ligature just below the atrium, on top of the LAD's main stem. Secure it with a suture on a silicon tube to maintain vessel integrity.

21. Place temporal sutures during ischemia between the ribs and use a compress to prevent tissue drying.

22. After the ischemia period, remove the silicon tube, cut the sutures, and observe reperfusion.

23. Supplementary 0.5% isoflurane can be administered in addition to the used anesthetic and analgesics.

2.5. Chemical Agent-Induced Myocardial Infarction Models

2.5.1. Isoproterenol-Induced Myocardial Infarction Models

Acute myocardial infarction induced by isoproterenol (ISO) is a non-surgical animal model developed in rats (34,35). Among experimental animal models, isoproterenol (ISO) is commonly used and best reflects the symptoms of MI in humans. Isoprenaline, also known as isoproterenol, is a non-selective β-adrenergic receptor agonist that induces MI at high doses (36). Isoprenaline stimulates the peroxidation of membrane phospholipids known to cause serious damage with increased auto-oxidation of the myocardial membrane, generating cytotoxic ROS. Therefore, it is widely used to induce MI in rats (37). The formation of ischemic necrosis is similar to that in humans. ISO causes necrosis in the ventricular subendocardial region and interventricular septum.

The reasons for choosing isoprenaline, especially for the myocardial infarction model, include its exceptional technical simplicity, repeatability, and an acceptable low mortality rate. ISO has been shown to induce ECG, biological, and histopathological changes in the MI model (36,38). Different doses of ISO are used in this model. In general, the literature reports doses of 65 mg/kg (39), 85 mg/kg (40,41), and 100 mg/kg (38,42).

In explaining this model, references were made to the studies by Kannan and Quine (35) and Beyazcicek and Beyazcicek (38).

2.5.1.1. Animals

Isoproterenol-induced myocardial infarction models are generally conducted using mice and rats. In the experimental application of this model, male rats weighing 250±30 grams and aged 2-3 months are commonly used as reference animals.

2.5.1.2. Experimental Procedure

The administration of isoproterenol is typically carried out in the last two days of the study, regardless of the duration of the study. Myocardial infarction in rats is induced by subcutaneous injection of 100 mg/kg isoproterenol hydrochloride dissolved in saline solution. Approximately 24 hours after the last administration, an increase in cardiac biomarkers such as CK-MB, cTn-T, and cTn-I levels is expected. EKG may show elevated or depressed ST-segment and the presence of a pathological Q-wave. Concurrently, oxidative stress parameters like MDA and total oxidative status may increase. Furthermore, histopathological changes can be observed.

2.5.2. Adrenaline-Induced Myocardial Infarction Models

Adrenaline, also known as epinephrine, is a stress hormone primarily produced by the adrenal glands and released into the bloodstream. It is part of the 'fight or flight' response and is a naturally occurring catecholamine in the body. It also has medical uses, including the treatment of cardiac arrest, allergic reactions, and asthma. However, it has been shown that at doses above physiological levels, adrenaline can lead to tissue damage by increasing the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (43). Myocardial infarction (MI) induced by adrenaline in rats is considered a reliable experimental model for studying the cardioprotective effects of drugs (44). Additionally, it has been discovered that adrenaline, as a contributing factor to MI, promotes lipid peroxidation and depletes cellular antioxidants (45). The description of this model is based on the work conducted by Aziz and colleagues (43).

2.5.2.1. Animals

Adrenaline-induced myocardial infarction models are typically conducted using rats. In experimental applications of this model, male Wistar albino rats weighing between 160-180 grams are commonly used as reference animals.

2.5.2.2. Experimental Procedure

In the case of adrenaline administration, similar to the isoproterenol model, it is applied during the last two days of the planned study. Myocardial infarction in rats is induced by subcutaneous injection of 1 mg/kg adrenaline dissolved in physiological saline. At the end of the study, changes in CK-MB, cTn-T, and cTn-I levels are observed, along with alterations in the ST-segment on the EKG. Additionally, there are increases in oxidative stress parameters such as MDA and total oxidative status. Furthermore, histopathological changes are observed.

3. In vitro Model of Myocardial Infarction

Animal models can be valuable resources for studying the progression of myocardial infarction (MI) and identifying potential treatment targets. However, the ability to test numerous potential treatments in animals is limited due to the use of complex surgical techniques. Additionally, the variability in functional outcome data and the necessity for time-consuming, sectioning, staining, and imaging of histopathological analyses may require a large collection of animal samples. Furthermore, genetic and physiological differences between small

animal models and humans can limit the usefulness of data in some cases. Therefore, as scientific knowledge advances and technology progresses, new modern developments that are hard to avoid are emerging. One of these is the newly developed organoid models.

Organoids are small, self-organized three-dimensional tissue cultures derived from stem cells. Such cultures can be produced to mimic a large part of the complexity of an organ or only reflect specific aspects, such as producing specific cell types. Organoids grow from stem cells that can self-renew and produce different cell types. Scientists have revealed how stem cells can follow their own genetic instructions to self-organize and create structures resembling miniature organs made up of many cell types. Organoid sizes can vary from the thickness of a hair to up to five millimeters (46). There are potentially as many types of organoids as there are various tissues and organs in the body. So far, researchers have successfully produced organoids resembling the brain, kidney, lung, intestine, stomach, liver, and even the heart.

Organoids have been used to study toxicology, genetics, environmental diseases, and cellular interactions, including engineered human heart tissues (46-50). Such engineered tissue models can assist in screening and discovering therapeutic agents for heart diseases. For this purpose, Ying Mei and colleagues have developed a human heart organoid myocardial infarction model (51).

3.1. Development of Human Heart Infarction Organoids

The development process of human heart infarct organoids was conducted by Richards et al. (51). In the description of this model summarized below, references were drawn from the studies conducted by Richards et al., as well as Mills and Hudson, and Richards et al. (51-53).

3.2. Cell Culture

Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are cultured according to the following producer protocol. This process consists of the following steps briefly:

1. Cardiomyocytes are placed in 6-well plates coated with 0.1% gelatin in iCell Cardiomyocyte Coating Medium (CDI).

2. They are incubated at 37°C in an environment with 5% CO_2 for 4 days.

3. On the third day, the coating medium is removed and replaced with 4 mL of CDI.

4. After the fourth day, the cells from the resulting monolayer pre-culture are detached using trypLE Express and used for spheroid/organoid production.

5. Human cardiac ventricular fibroblasts (cFBs) are cultured in FGM-3 medium.

6. Human umbilical vein endothelial cells (HUVECs) are cultured in EGM-2 medium.

7. Human adipose-derived microvascular endothelial cells (HAMECs) are cultured in EGM-2 medium.

8. Human adipose-derived stem cells (hADSCs) are cultured in low-glucose Dulbecco's modified Eagle medium containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% glutamine, and 1% antimycin.

9. The medium for cFBs, hADSCs, and HUVECs is changed every 2 days, and cells are passaged using trypLE Express when they reach >80% confluence.

3.3. Organoid and Spheroid Production

1. Non-adherent agarose hydrogel molds with hemispherical bases (800 μm in diameter, 800 μm in depth) containing 35 micro-wells are created to facilitate the formation of spherical micro-tissues.

2. For this purpose, negative replicas are made using 2% agarose and master micro-molds.

3. The molds are soaked in cell/organoid-specific medium before micro-tissue production.

4. Cell suspensions of each cell type are used at ~4.0×10^6 cells/mL to prepare mixtures for organoid cell ratios.

5. Cells are mixed with 1 volume of medium to achieve a concentration of ~2.0×10^6 cells/mL.

6. Approximately 75 μL of cell suspension is pipetted into each agarose mold.

7. After cells have settled into the mold cavities (for 10 minutes), additional medium is added to immerse the molds in an 8-well plate.

8. The medium is changed every 2 days throughout the working period.

9. Experiments begin after four days of spheroid formation."

3.4. Cardiac Organoid Infarction Protocol

1. Micro-tissues for cardiac organoid infarction are placed in a hypoxic environment containing 1 μM noradrenaline in 10% O_2 for a duration of 10 days (~150 μm radius).

2. The medium is changed every 2 days (with noradrenaline) throughout the experiment.

3. At the end of the study, L-lactate levels in the medium of both control and infarcted organoids are measured using lactate assay kits.

4. Conclusion

Experimental myocardial infarction models are crucial research tools that aid scientists and researchers in gaining a better understanding of the causes, mechanisms, and treatment methods of myocardial infarction. These models enable us to investigate and comprehend the underlying pathophysiological processes of myocardial infarction. Furthermore, they contribute to the development of treatment methods, the testing of the reliability and effectiveness of potential drugs, genetic research, and the examination of revascularization strategies. In conclusion, experimental myocardial infarction models play a critical role in advancing scientific knowledge related to heart attacks, developing new treatment options, and making significant progress in the fight against heart disease in the field of healthcare.

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CHAPTER V

MENINGES AND DURAL SINUSES

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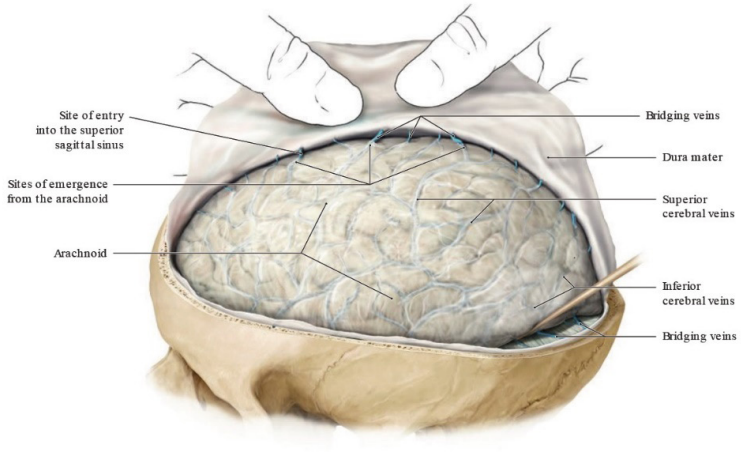
1. Introduction

The meninges, which are referred to as the meninx primitiva during the embryonic period, are divided into two groups: the endomeninx, which is the tough membrane, and the ectomeninx, which is the soft membrane. (1, 2) In later stages, the dura mater develops from the ectomeninx, while the arachnoid and pia mater develop from the endomeninx. (1) The brain and spinal cord are enveloped from the outside inward by the membranes known as the dura mater, arachnoid mater, and pia mater. (1, 3, 4) These membranes surrounding the brain are called the cranial dura, arachnoid, and pia mater, while the portion enveloping the spinal cord is referred to as the spinal dura, arachnoid, and pia mater. (1, 3-5)

1.1. Dura mater

Consisting of connective tissue with collagen fibers, the dura mater is a thick and non-elastic structure. (1, 2, 4, 5) The cranial dura mater is composed of two layers: the periosteal dura and the cerebral dura. The periosteal dura, adhering to the bone, is called the lamina externa, while the cerebral dura facing the brain is referred to as the lamina interna (Figure 1). (1, 2, 4, 5)

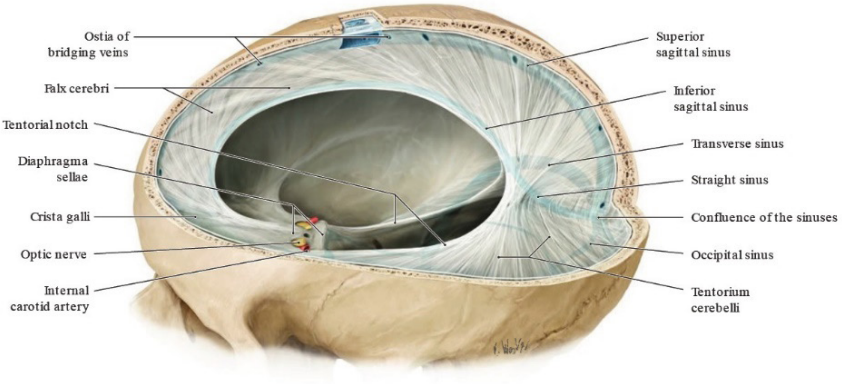
Figure 1. Dura and arachnoidea mater



Source: "THIEME Atlas of Anatomy". (6)

Although these two layers are normally fused, they separate from each other in some areas to form the dural sinuses that collect venous blood from the brain. (1-4) The dura mater on the calvarium can be easily detached from the bone, while it is more difficult to separate it from the bone on the cranial base. (7) The lamina interna sends projections called dural septa towards the brain, which protect the cranium from external mechanical forces, stabilize organs, shape the interior of the cranium, and separate certain parts of the brain from each other. (2) These extensions are known as the falx cerebri, falx cerebelli, tentorium cerebelli, and diaphragma sellae (Figure 2). (1-8)

Figure 2. Dura mater extensions and dural sinuses



Source: "THIEME Atlas of Anatomy". (6)

1.1.1. Falx cerebri

Anteriorly, the falx cerebri is attached to the crista galli, and posteriorly, it is anchored to the protuberantia occipitalis interna, coursing within the fissura longitudinalis cerebri. (1, 2, 5) Superiorly, it is attached to the superior sagittal sinus, extending to the cranium, and inferiorly, it extends to the corpus callosum, with the inferior sagittal sinus situated at its lower free end (Figure 2). (2, 5)

1.1.2. Tentorium cerebelli

The tentorium cerebelli is affixed to the protuberantia occipitalis interna posteriorly, and to the sinus transversus and pyramis laterally, progressing anteriorly where it is attached to the anterior and posterior clinoid processes. (2) At the point of attachment to the pyramis, the superior petrosal sinus is located between the two laminae. (3) Towards the posterior attachment on the occipital bone, the sinus transversus is located. (3) The falx cerebri is superiorly attached to the middle of the tentorium cerebelli, where the falx cerebelli is attached inferiorly, forming the straight sinus (Figure 2). (3)

This membrane that separates the cerebellum from the occipital lobe of the cerebrum, covering the cerebellum like a tent, is divided into two regions: the supratentorial region above, and the infratentorial region below. (4) As it extends anteriorly, a notch known as the incisura tentorii is formed between its two arms. Through this notch, the mesencephalon, vessels, and some cranial nerves pass. (2, 5)

Growths occurring in the supratentorial region such as tumors can lead to increased intracranial pressure, and a portion of the temporal lobe from neighboring structures can herniate through this notch. In this condition known as tentorial herniation, the oculomotor nerve can be affected, resulting in paralysis of the eye muscles innervated by the oculomotor nerve. (7)

1.1.3. Falx cerebelli

The falx cerebelli is a short, sickle-shaped dura segment that lies between the two cerebellar hemispheres, attaching to the crista occipitalis interna superiorly (Figure 2). (2, 5) The occipital sinus is located between its two laminae. (1)

1.1.4. Diaphragma sellae

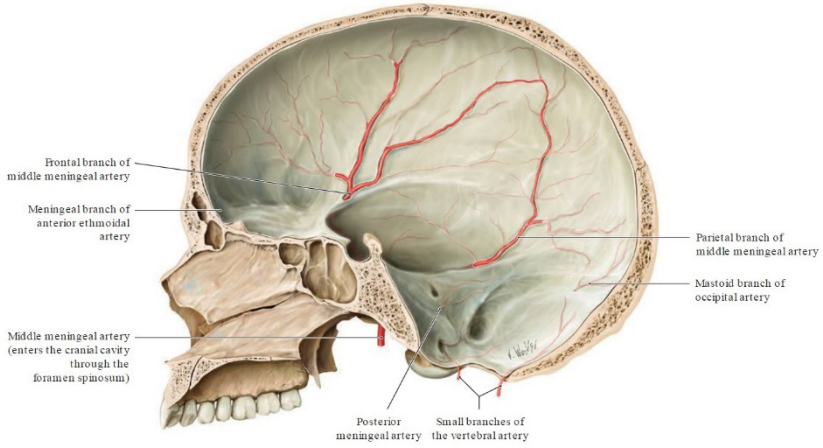
This dural septum, which is attached to the anterior and posterior clinoid processes and runs transversely, covers the sella turcica (fossa hypophysialis). (2, 4, 5) Within this dural extension, there is an opening for the passage of the infundibulum hypophysialis. (2, 4, 5)

The spinal dura mater begins at the foramen magnum and ends by closing off around the level of the second sacral vertebra (S2). (4, 5) As with the cranial dura mater, it consists of two layers, and the space between them is referred to as the epidural space. (1, 5) The two layers fuse into the filum terminale externum at the level of the S2 vertebra and extends to its attachment on the periosteum of coccyx. (4, 5)

1.1.5. Blood vessels and innervation of the dura mater

The middle meningeal artery, a branch of the maxillary artery, enters the cranial cavity through the foramen spinosum and runs laterally within the middle cranial fossa. Here, the artery splits into anterior and posterior branches. The anterior branch advances towards the pterion and then ascends posteriorly along the cranial base. Trauma to the lateral skull in the pterion region can potentially result in damage to the middle meningeal artery. The posterior branch of the middle meningeal artery divides into branches in the posterior area of the skull, supplying blood to this region (Figure 3). The dura mater is also supplied by small branches emerging from the ophthalmic, occipital and vertebral arteries. (5-8)

Figure 3. Vessels of the dura mater and middle meningeal artery

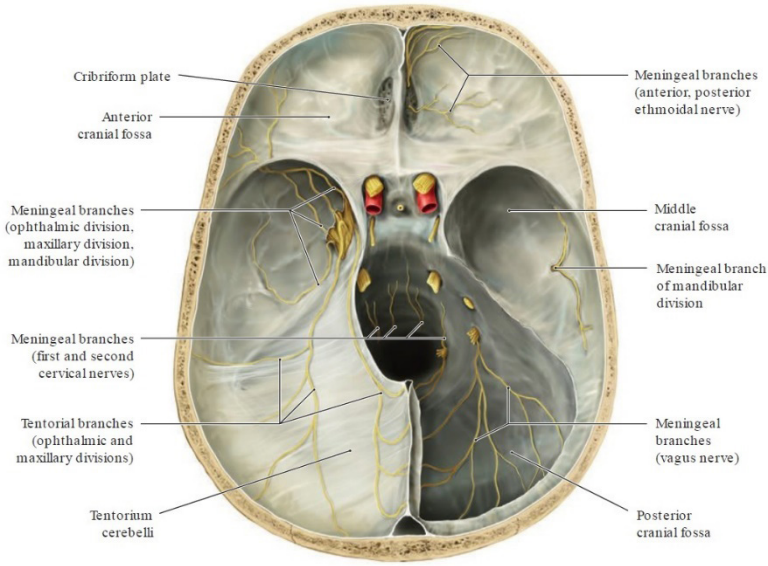


Source: "THIEME Atlas of Anatomy". (6)

The veins of the dura mater run along the middle meningeal artery. They exit the cranium through the foramen spinosum or foramen ovale and drain into the pterygoid plexus. (5, 7, 8)

The anterior and middle compartments of the cranial fossa are primarily innervated by the branches of the trigeminal nerve. The dura of the anterior cranial fossa is innervated by the meningeal branches from the three divisions of the trigeminal nerve. The dura of the middle cranial fossa is innervated by the branches of the maxillary and mandibular divisions of the trigeminal nerve, and the dura of the posterior cranial fossa is innervated by the branches from the vagus nerve, the branches of the ophthalmic division (n. ophthalmicus), and the sensory branches of C1-C3 (Figure 4). (5-8)

Figure 4. Nerves that provide innervation of the dura mater



Source: "THIEME Atlas of Anatomy". (6)

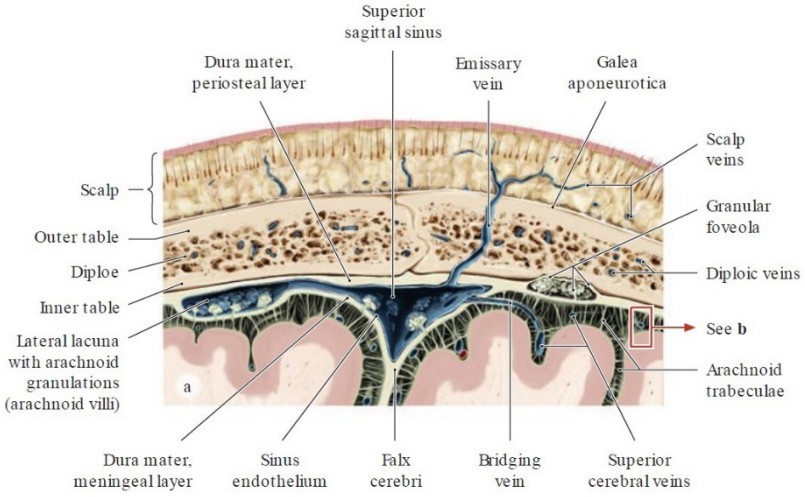
1.2. Arachnoidea mater

The arachnoid mater is a delicate but impermeable membrane located between the dura mater and the pia mater (Figure 1). (2, 3, 5) The arachnoid mater is avascular and therefore, it is supplied by the cerebrospinal fluid (CSF). (4) The potential space formed between the arachnoid mater and the dura mater is called the subdural space, and the space between the arachnoid mater and the pia mater is referred to as the subarachnoid space. (3, 5) The subdural space contains a small amount of serous fluid, while the subarachnoid space contains CSF. (3) The cranial arachnoid mater covers cerebral gyri but does not extend

into the sulci, whereas the spinal arachnoid mater envelopes the inner portion of the spinal dura mater and terminates at the level of the S2 vertebra along with the spinal dura mater. (4, 5)

The cranial arachnoid mater possesses small villus-like protrusions on its dura mater-facing side. (2, 4) These projections come together to form cauliflower-like structures known as arachnoid granulations that invaginate into the dural sinuses, aiding in the drainage of CSF into the venous circulation (Figure 5). (2, 4, 5) They are usually found in the superior sagittal sinus and occassionally in the transverse sinus. (4) In the spinal cord, these granulations are found in areas close to the brain where the spinal nerve roots are present. (2)

Figure 5. Illustration of arachnoid granulations



Source: "THIEME Atlas of Anatomy". (6)

The arachnoid mater is thicker in the lower parts of the brain, and since the pia mater invaginates into the cerebral compartments while the arachnoid mater bridges across the gyri and sulci, this leaves wider spaces between them, forming the subarachnoid cisterns. (3, 5) Major cisterns are described below in detail (Figure 6):

1.2.1. Cisterna cerebellomedullaris posterior (Cisterna magna)

It is the largest cistern which is situated between the lower part of the cerebellum and the dorsal aspect of the medulla oblongata. (4, 5) Cerebrospinal fluid passes through the fourth ventricle into the cisterna magna via the median

aperture (foramen of Magendie). Within the medulla spinalis, the cisterna magna continues with the subarachnoid space, and within the cranium, it is connected to the lateral cerebellomedullary cistern. (3, 5) The lateral cerebellomedullary cistern contains the vertebral artery, the glossopharyngeal, vagus and accessory nerves, and anterolateral medullary veins. (4)

1.2.2. Cisterna interpeduncularis (Cisterna basalis)

The interpeduncular cistern is the space between the two temporal lobes. It is bounded by the mesencephalon superiorly, lower part of the diencephalon, corpus mamillare, the posterior perforated substance, and the clivus antero-inferiorly. (3-5) Laterally, this space continues with the cisterna ambiens, and contains the circulus arteriosus cerebri (circle of Willis), oculomotor nerve and basal vein. (4)

1.2.3. Cisterna ambiens (Cisterna superior)

The ambient cistern is situated at the lateral sides of the mesencephalon, between the tectum mesencephali and the lobus quadrangularis of the cerebellum. (3-5) It connects the cisterna quadrigeminalis to the cisterna interpeduncularis and also neighbors the cisterna pontocerebellaris inferiorly. (3, 4) It contains the great cerebral vein (v. magna cerebri), corpus pineale, and posterior and superior cerebellar arteries. (4)

1.2.4. Cisterna pontocerebellaris

This cistern lies between the anterior surface of the cerebellum and lateral surface of the pons (3, 4) It continues with the subarachnoid space inferiorly in the medulla spinalis and the cisterna interpeduncularis superiorly. It contains the internal carotid artery, anterior inferior cerebellar artery, petrosal vein, and the trigeminal, facial and vestibulocochlear nerves. (3, 4)

1.2.5. Cisterna fossae lateralis cerebri

In this space formed between the sulcus lateralis cerebri and the arachnoidea mater cerebri, the following arteries are located: the middle cerebral artery, lenticulostriate artery, and anterior temporal artery (3, 4)

1.2.6. Cisterna chiasmatica

This cistern continues below with the cisterna interpeduncularis and encircles the chiasma opticum. It contains the internal carotid artery, anterior cerebral artery, middle cerebral artery and anterior choroidal artery. (3, 4)

1.2.7. Cisterna pericallosa

This cistern is located above the corpus callosum, between the gyrus cinguli and the corpus callosum and continues with the cisterna lamina terminalis anteriorly and the cisterna quadrigeminalis posteriorly. (3, 4)

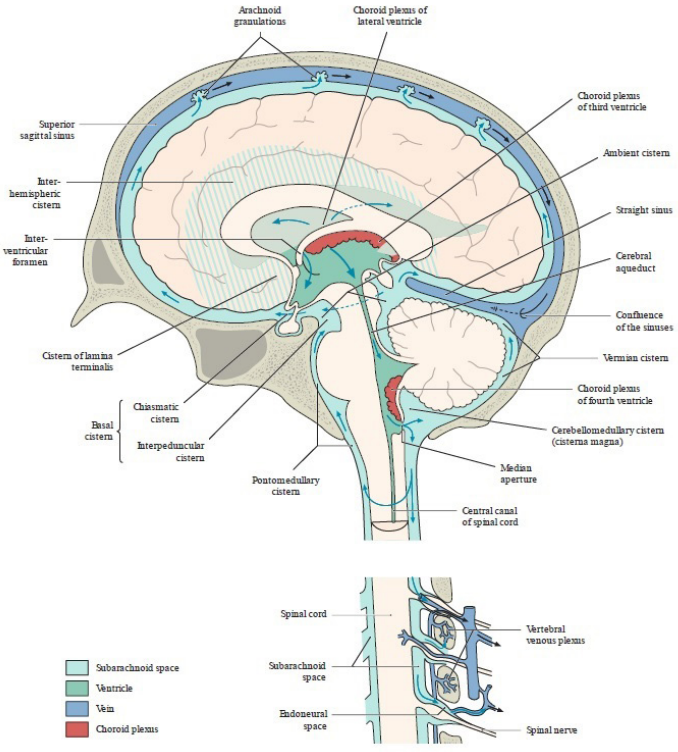
1.2.8. Cisterna quadrigeminalis

This is the space situated between the splenium of the corpus callosum, the tectum mesencephali, and the lingula vermis cerebelli. (3, 4) Within this cistern, the great cerebral vein, pineal gland, posterior cerebral artery, posterior communicating artery, basal vein, and occipital vein is are located. (4)

1.2.9. Cisterna lumbalis

It is the expansion located between the L2 vertebra and the S2 vertebra at the lower part of the medulla spinalis. (4) This space, where the filum terminale internum and the structures of the cauda equina are found, is the region where lumbar puncture is performed for CSF collection. (4)

Figure 6. Subarachnoid cisterns and the course of CSF



Source: "THIEME Atlas of Anatomy". (6)

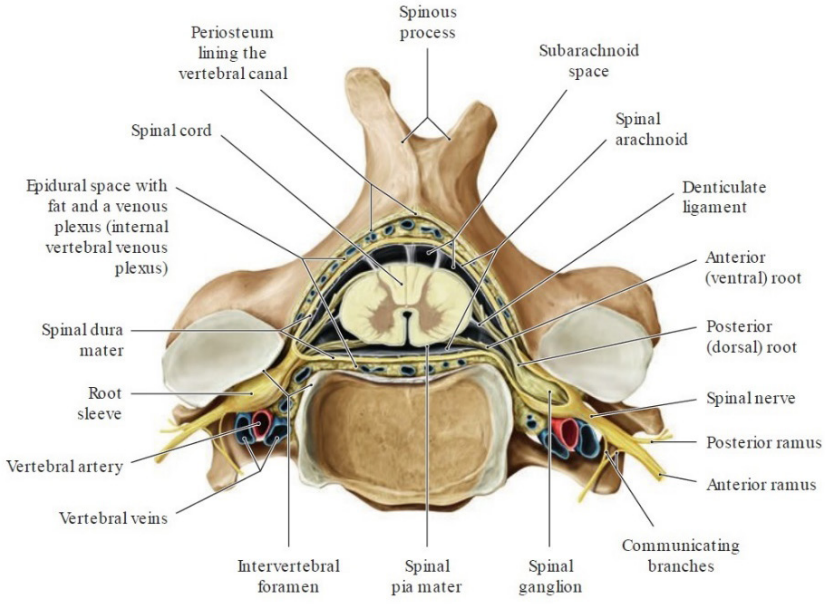
1.3. Pia mater

It is a delicate fibrous membrane that closely envelopes all sulci and fissures of the cerebral hemispheres and the medulla spinalis. (1-5) With its abundant blood vessels, the pia mater supplies the brain and the medulla spinalis, and penetrates the cerebral tissue around the vessels. (3) Additional fibers from the pia mater also pass through the dura mater, intertwining with its fibers, and reach the perineurium layer around the cranial and spinal nerves emerging from the brain and medulla spinalis. (3, 5)

The pia mater cranialis invaginates into the areas where the ventricles are located, and it is encircled by the ependymal cells lining the ventricles, resulting in the formation of the tela choroidea consisting of two layers of the pia mater. (3-5) The choroid plexus is found between the two sheets of the pia mater. It secretes CSF and is supplied by the branches of anterior and posterior choroidal arteries. (4, 5)

The pia mater spinalis terminates at the lower end of the medulla spinalis and extends down to the level of the S2 vertebra, forming a structure called the filum terminale internum along with ependymal cells, within a connective tissue rich in collagen fibers. At this point, it merges with the dura mater and arachnoidea mater to form the coccygeal ligament (Figure 7). (4, 5)

Figure 7. The spinal cord meninges
(Transverse section, view inside the vertebral canal)



Source: "THIEME Atlas of Anatomy". (6)

The pia mater spinalis is stronger, thicker, yet less vascularized compared to the pia mater cranialis. (3) It consists of two layers: an outer longitudinal layer and an inner circular layer. (3) The circular layer adheres tightly to the medulla spinalis and enters through the fissura mediana anterior. The longitudinal layer thickens along the sides of the medulla spinalis where it is attached to the arachnoidea mater and dura mater, forming the denticulate ligaments which stabilize the medulla spinalis (Figure 7). (3-5, 8) This ligament has a notched appearance on its outer side and is anchored to the dura mater spinalis through the ventral and dorsal roots of each spinal nerve at a total of 21 locations, from the foramen magnum to where the first lumbar spinal nerve emerges. (3)

2. Clinical significance of the meningeal spaces

The spatium epidurale cranialis is the space between the cranium and the dura mater. (1-4) In the case of a head injury, bleeding can occur in the middle meningeal artery, which supplies the dura mater, or ruptures can develop in the veins within the epidural space or the diploic veins, leading to epidural hematomas. (4) Depending on the location of the bleeding, a range of symptoms can occur due to pressure on various parts of the brain, and these symptoms can be life-threatening. Therefore, surgical intervention is required for the treatment of epidural hematomas. (4)

In the spatium epidurale spinalis, the plexus venosus vertebralis internus forms anastomoses with the plexus venosus vertebralis externus. These venous plexuses are connected at various levels with v. azygos, vv. lumbales, and vv. cervicales profundi. When intra-abdominal pressure rises due to conditions such as pelvic, lung, or abdominal tumors or infections, these connections can facilitate the spread of these tumors or infections to the central nervous system. (4)

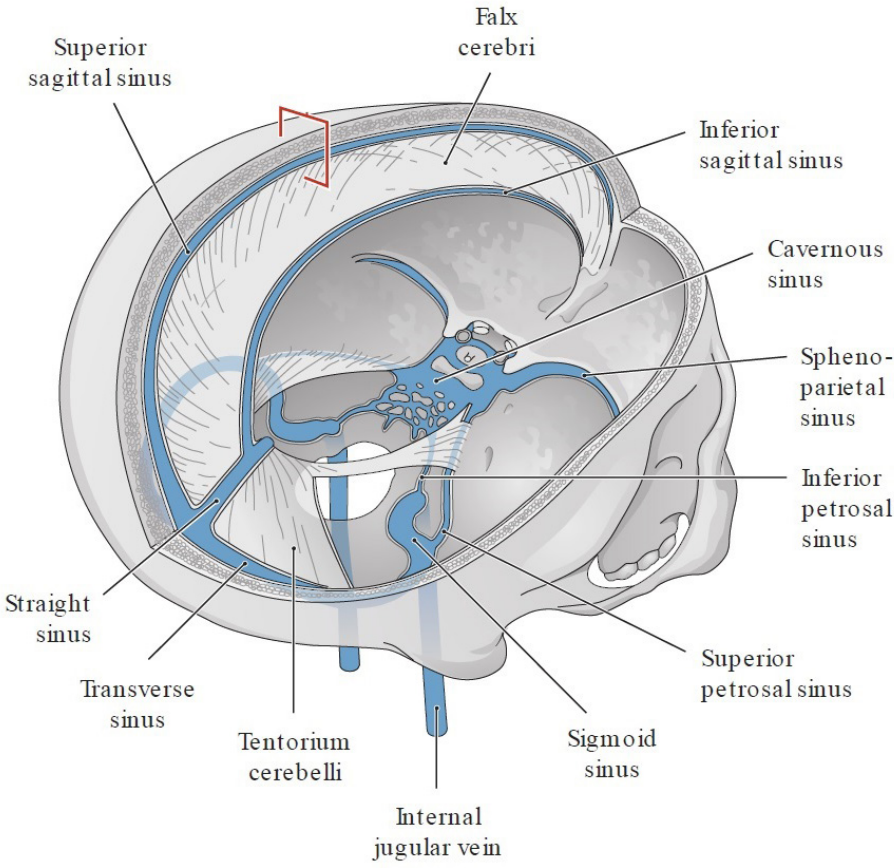
When the CSF pressure increases, it can pass from the spatium subarachnoideum, where it normally resides, to the spatium subdurale. In older individuals, children, substance abusers, or in cases of accidents or trauma, bleeding from cortical veins and pial arteries can accumulate in the subdural space. This condition can lead to short-term loss of consciousness. (4)

Subarachnoid hemorrhages usually stem from aneurysms in the blood vessels of the central nervous system. In such cases, sudden and severe headaches, loss of consciousness, stroke, and even respiratory arrest can occur. (4)

3. Dura mater sinuses

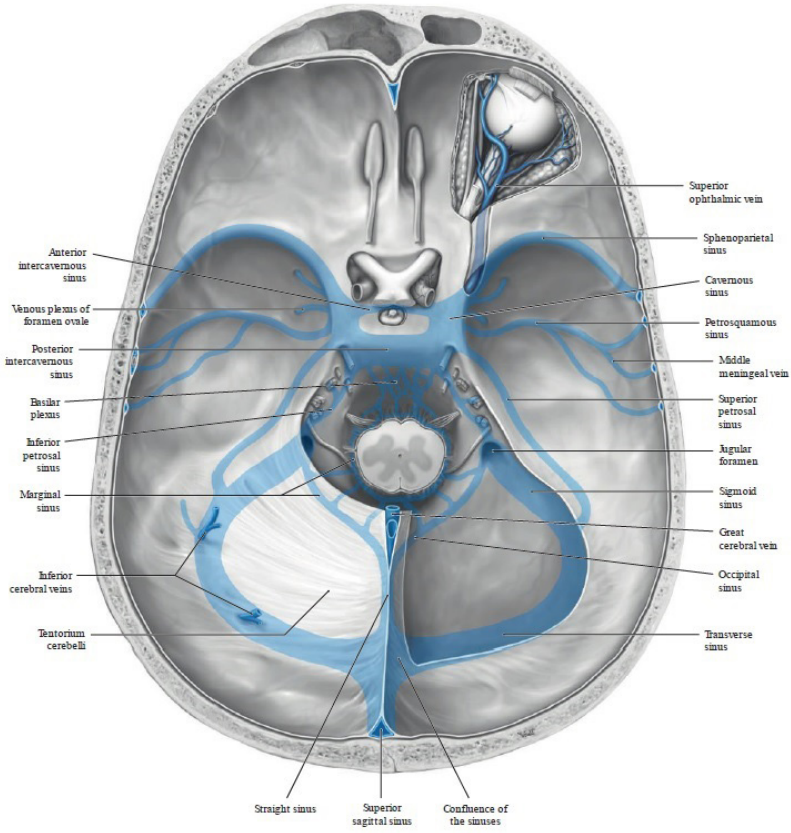
These spaces located between the two layers of the dura mater encephali drain blood and CSF from the brain and the cranial bones. The inner surface of the dura mater sinuses is lined with endothelium, and they lack a lumen and smooth muscle in their walls (Figures 8, 9). (1-5)

Figure 8. Illustration of dural sinuses



Source: "THIEME Atlas of Anatomy". (6)

Figure 9. Location of the dural sinuses in the base of the skull

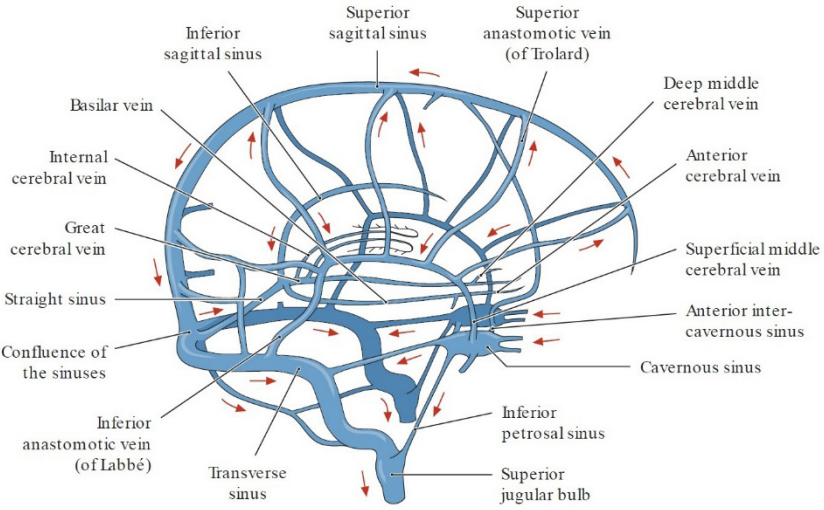


Source: "THIEME Atlas of Anatomy".

3.1. Sinus sagittalis superior

The superior sagittal sinus extends from where the falx cerebri is attached to the calvaria around the crista galli anteriorly to the protuberantia occipitalis interna posteriorly. There, it widens to form the confluens sinuum. Then, it continues as the right transverse sinus (in 60% of humans) or the left transverse sinus (in 40% of humans) (Figure 10). It is the largest dural sinus draining the superficial cortical veins and the superior anastomotic vein. The superior sagittal sinus also serves as the primary conduit for CSF transfer from the subdural space to the dural sinuses. Consequently, obstruction of this space may lead to impaired venous return and compromised CSF reabsorption, resulting in increased intracranial pressure. (1-5, 7, 8)

Figure 10. A drawing illustrating the course of CSF circulation and the dural sinuses.



Source: "THIEME Atlas of Anatomy". (6)

3.2. Sinus sagittalis inferior

The inferior sagittal sinus extends posteriorly from the inferior free edge of the falx cerebri to the anterior edge of the tentorium cerebelli. There, it merges with the v. magna cerebri, also known as the vein of Galen, to form the sinus rectus (Figure 10). (1-5, 8)

3.3. Sinus rectus

Located at the junction of the falx cerebri and the tentorium cerebelli, this sinus is formed by the confluence of the v. magna cerebri and the sinus sagittalis inferior. It continues posteriorly and opens into the confluens sinuum, typically advancing to the left side and opening into the transverse sinus (Figure 10). (3, 5-8)

3.4. Sinus occipitalis

The occipital sinus, sometimes observed in pairs, is the smallest dural sinus found where the falx cerebelli is attached to the occipital bone. It is formed by the convergence of veins around the foramen magnum and runs upward to drain into the confluens sinuum (Figure 10). Since it is connected with the plexus venosus vertebralis internus and lacks valves, it can potentially facilitate the spread of extracranial infections or malignant cells into the cranium. (2-5, 7, 8)

3.5. Sinus transversus

The transverse sinus, situated where the sulcus sinus transversus attaches to the tentorium cerebelli, is formed by the confluence of the superior sagittal sinus and the straight sinus at the confluens sinuum. The superior sagittal sinus usually ends becoming the right transverse sinus, while the straight sinus is mostly continues with the left transverse sinus. The inferior cerebral veins as well as inferior cerebellar veins drain into this sinus. (2-5, 7, 8)

3.6. Sinus sigmoideus

Continuing with the transverse sinus, this sinus extends downward and medially in an S-shaped pattern through the layers of the tentorium cerebelli, progressing towards the foramen jugulare (Figure 10). It courses within the sulcus sinus sigmoideus, a channel found in the parietal, temporal, and occipital bones. Within the jugular fossa, it merges with the bulbus vena jugularis interna and continues as the v. jugularis interna after passing through the foramen jugulare, along with the cranial segments of n. glossopharyngeus, n. vagus, and n. accessorius. (2-8)

3.7. Sinus cavernosus

On both sides of the corpus of the os sphenoidale, there are cavernous dural sinuses. Commencing from the superior orbital fissure and extending to the pyramis, two cavernous sinuses on the right and left sides communicate by way of anterior and posterior intercavernous sinuses, located in front of and behind the pituitary gland. This connection results in the formation of a venous ring around the diaphragma sellae. (2-5, 7, 8)

Collecting the venous blood of structures neighboring the sulcus lateralis in the cerebral hemispheres, v. media superficialis cerebri and the vein of Sylvian drain into the sinus cavernosus through the sinus sphenoparietalis. The sinus occipitalis inferior and sinus temporalis lateralis also open into the sinus cavernosus. Additionally, the superior and inferior ophthalmic veins and the central retinal vein carry venous blood from the orbital region to the sinus cavernosus. Considering the connection between the facial vein and the ophthalmic vein, any infection on the facial skin can reach the sinus cavernosus through this venous connection, potentially affecting the cerebral meninges. (2-5, 7, 8)

The sinus cavernosus drains into the sinus transversus through the sinus petrosus superior and into the v. jugularis interna through the sinus petrosus inferior. (4, 7)

3.7.1. Structures passing through the sinus cavernosus

The structures passing through the sinus cavernosus are clinically significant. The sympathetic nerve plexus surrounding the internal carotid artery, the trochlear and abducens nerves pass through the sinus cavernosus. Also, the oculomotor nerve, trochlear nerve, ophthalmic nerve, and maxillary nerve travel between the endothelial lining and the dura matter of the lateral wall of the cavernous sinus. (2-5, 8)

In cerebral venous thrombosis affecting the sinus cavernosus, these nerves can be involved, leading to a variety of neurological symptoms. Thrombosis generally occurs as a result of infections such as those affecting the midface, sphenoid and ethmoidal paranasal sinuses, as well as dental infections, and otitis media. Despite being rare, thrombosis has a high mortality rate. (4)

3.8. Sinus petrosus superior

It is a thin dural sinus connecting the sinus cavernosus to the sinus transversus, and runs along the upper margin of the pyramis. (3, 8)

3.9. Sinus petrosus inferior

It travels along the lower border of the pyramis and connects the sinus cavernosus to the internal jugular vein. The labyrinthine vein drains into this sinus. (3, 8)

The sinus petrosus inferior communicates with the plexus basilaris located between the two sheets of the dura mater over the clivus behind the dorsum sellae, and with the plexus venosus vertebralis. It also connects the sinus petrosus superior and sinus cavernosus to each other. (3, 7)

3.10. Sinus sphenoparietalis

This sinus is situated along the free posterior edge of the ala minor of the os sphenoidale. It drains into the sinus cavernosus. (3)

4. Conclusion

- The cerebrum and medulla spinalis are enveloped by three layers from superficial to deep, which are the dura mater, arachnoid mater, and pia mater.
- The space between the dura mater and arachnoid mater is called the subdural space, while the space between the arachnoid mater containing cerebrospinal fluid and the pia mater is known as the subarachnoid space. The subarachnoid space expands in certain areas to form cisterns.

• The structures known as “arachnoid granulations” found in the arachnoidea mater cranialis are involved in transporting CSF to the venous system.

• The cranial pia mater forms invaginatons in the ventricular region. These invaginations as well as ependymal cells that line the cerebral ventricles form the choroid plexus together with the branches of the anterior and posterior choroid arteries.

• The pia mater spinalis terminates at the end of the medulla spinalis and forms an extension called the filum terminale internum that extends downward within the dura mater. At the S2 level, it joins with the arachnoid mater and dura mater, becoming the coccygeal ligament to attach to the back of the coccyx.

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CHAPTER VI

THE ROLE OF THE APELINERGIC SYSTEM IN DISEASES

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1. Introduction

Apelin (APLN) is an adipokine produced primarily by white adipose tissue and was first identified in bovine stomach extracts (1). APLN is widely distributed in various organs in humans and animals (2). APLN and APLN receptor (APJ) are common in the brain, heart, lung, liver, kidney, gastrointestinal tract, endothelium, and fatty tissues (3). Recent studies draw attention to the relationship of APLN/APJ with diseases, and among these, the potential to inhibit the inflammatory response is important (2). The APLN/APJ system has various functions in rodents and humans, ranging from fluid homeostasis to regulation of energy metabolism (4). Studies say that the increase in APLN may have a dual structure. For example, While it can positively affect angiogenesis after ischemic stroke, it can also stimulate angiogenesis in cancer (5, 6). Therefore, the APLN/APJ system attracts attention as a possible treatment target in different pathologies (4).

2. Discovery of Apelin and Apelin Receptor

The extant story of APLN began in 1993 with the cloning of a cDNA for an orphan receptor called the "APJ receptor" (the putative receptor protein related to the type 1 (AT1) angiotensin receptor) from the human genome library. Although APJ is 31% similar in amino acid sequence to the human AT1 receptor and 54% identical in its hydrophobic transmembrane region, it does not

bind to members of the angiotensin family (7, 8). APLN and AT1 receptors are coexpressed in the cardiovascular system. APJ is well conserved across species, with nearly 90% sequence similarity between mouse, rat, and human proteins (9). The APJ receptor, orphaned until 1998, found its ligand when Tatemoto and colleagues identified a 36-amino-acid endogenous peptide called APLN (10). APLN is an endogenous ligand of the APJ, a seven-transmembrane G protein-coupled receptor (11, 12).

The APLN gene is settled on chromosome Xq25-q26.3. It codes a 77 amino acid prepropeptide and can be cleaved into different active types, such as APLN-36, APLN-17, APLN-13, and APLN-12 (1). APLN-36 is the most widely expressed, while APLN-13 is more dynamic and frequently found in circulation (13). APLN, a vasoactive peptide, is prominent in managing body fluid homeostasis and cardiovascular roles. According to studies conducted in rodents, APLN has aquatic effects that occur through the central nervous system and kidney (14).

APLN activity may be modulated by interactions of the APJ receptor with other receptors, resulting in heteromerization (15). The APLN system includes the APLN receptor and its two endogenous ligands, APLN and apela/elabela (ELA; also known as APLN receptor early endogenous ligand)/toddler (16, 17). Although predicted isoforms ELA-32, ELA-21, and ELA-11 bind to the APLN receptor in the human heart, ELA peptides show little sequence similarity to APLN (18). ELA also supports vasodilation, but its mechanism can be different than APLN. Apela is thought to be present in the adult heart, regulating post-infarction cardiac remodeling and enhancing cardiac contractility in an ERK1/2-dependent manner (17). It has been suggested that APLN regulates cardiac hypertrophy through various pathways and that the endogenous ligand ELA may alleviate cardiac hypertrophy through similar or different mechanisms than APLN (19).

The emergence of ELA as another ligand for the APLN receptor has led to the elucidating of the phenotypes of APJ knockout mice that do not conform to Mendelian ratios and have cardiovascular developmental defects (20). Cardiovascular malformations appear to be increased in APLN receptor-deficient mice (21). APLN knockout mice showed a propensity for injury with increased infarct area, ventricular dilatation, and mortality compared to the controls (22). APLN reduces the entry and oxidation of fatty acids into the myocardium in rodents (23).

3. Apelinergic System in COVID-19

There is evidence that APLN improves lung injury and coagulopathy in COVID-19 (24). Recent COVID-19 studies confirm that hypertension and some cardiovascular disorders are seen in patients who develop acute respiratory distress syndrome (25). It is thought that APLN may play a positive role in the process by which SARS-CoV-2 binds to and downregulates ACE2 and activates Ang-II-mediated pathological pathways in endothelial and epithelial cells in the lung, heart, and vascular system (26). APLN 12 and 13 can suppress the vasoconstrictive effect of Ang-II and provide positive cardiovascular effects via L-arginine/endothelial nitric oxide synthase (27). Elevated D-dimer and fibrin degradation products are observed in deaths from COVID-19, and thromboembolism with disseminated intravascular coagulation is observed. It is hypothesized that APLN or APJ agonists may help correct abnormal coagulation parameters (24, 25, 28).

4. The Role of the Apelinergic Axis in the Neurodegenerative Diseases

Neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's, are incurable diseases characterized by progressive loss of cognitive or motor function that impair the quality of life in the aging population. The physiological role of the APLN/APJ axis appears to be closely related to the emergence of neurodegenerative diseases (29). APLN is distributed in neuronal cell bodies and the hypothalamic area (30). Additionally, APLN alleviates nerve damage caused by drugs of abuse and contributes to the reduction of oxidative stress, apoptosis, and autophagy (31). The apelinergic system may alleviate acute brain injury. Additionally, its positive effects on neuroinflammation are mentioned (32). There is data that APLN-13 inhibits LPS-induced neuroinflammation and improves cognitive impairment (33).

APLN mediates endoplasmic reticulum stress reduction and neuroinflammation attenuation through AMPK inhibition (34). The APLN/APJ system suppresses p-tau production by inhibiting inflammation through the BDNF/TrkB axis. This emphasizes the importance of the APLN/APJ system in Alzheimer's patients (32). Expression of APLN appears to be increased in epileptic experimental models (35). While apelinergic signaling aggravates PTZ-induced epileptic seizures, APLN 13 treatment inhibits neuroinflammation with positive effects on astrocytes (36). It has also been observed that the APLN/APJ system is activated in inflammatory and neuropathic pain (37). APLN is evaluated as a marker of angiogenesis in glioblastoma-like tumors (38). It was

reported that the tumor vascular area in the APLN knockout mouse brain was reduced, and the survival rate of the mice was increased (39).

5. The Role of the Apelinergic Axis in the Digestive System Diseases

Histochemical methods revealed an APJ/APLN system in digestive tract cells. It is thought to regulate many physiological processes, including apoptosis (40). Apoptosis is closely associated with many developmental processes, and any dysfunction can lead to cancer, autoimmune diseases, and developmental defects (41). APLN has functions such as cell proliferation in the digestive system and controlling gastric and pancreatic secretions. Additionally, APLN is thought to have a gastroprotective effect by protecting the gastric mucosa (42). The apelinergic system has the potential to contribute positively to the healing of stress-induced gastric damage and the prognosis of gastric cancer. It also plays an important role in reducing the inflammatory response (43).

6. The Role of the Apelinergic Axis in the Cancer

The APLN/APJ axis is thought to have a role in lung cancer, gastrointestinal cancers, genitourinary system cancers, oral squamous cell carcinoma, and brain cancer. Its function in tumor neoangiogenesis is particularly noteworthy (11). It is reported that APLN is increased in many types of cancer. The APLN/APJ axis is involved in tumor development by increasing angiogenesis, metastasis, and proliferation (44). It has been reported that APLN expression is associated with tumor size, stage, histological type, lymph node metastasis, and poor prognosis and may have predictive value for breast cancer (45). Studies show that the Apelinergic system in cancer cells negatively regulates epithelial-mesenchymal transition and tumor malignancy (46). The presence of a functional APJ is thought to be important for successful cancer immunotherapy (47).

7. The Role of the Apelinergic Axis in the Kidney Diseases

It is predicted that plasma APLN concentrations may decrease with age and affect the functions of many organs, including the kidneys (48). APLN is observed in endothelial cells in the kidney and adrenal glands (49). Chronic kidney disease is an increasing public health problem and can cause permanent impairment of kidney function (50). Despite treatment options, many patients progress toward kidney failure (16). Kidney diseases are also associated with cardiovascular diseases (51).

Hypertension may develop due to chronic renal failure (52). The mainstay of chronic kidney disease treatment is the renin-angiotensin-aldosterone system's blockade. Studies in healthy volunteers show reciprocal changes in vasopressin and APLN levels (53). Mice with APLN gene deficiency chronically exposed to excessive pressure develop severe heart failure (54). Modifying the C-terminal residue of APLN 13 may promote the hypotensive effect of APJ (2). Knockout of the prorenin receptor in mice increased APLN and ELA mRNA levels (55). Although APLN 13 and ELA have different mechanisms of action, it is suggested that the combined treatment has a synergistic effect (56). It is thought that the apelinergic axis may improve renal interstitial fibrosis. In addition, this axis prevents some dialysis complications. It can alleviate vascular calcification. The Apelinergic axis may play different roles in kidney diseases and provide hope for treatment (57).

8. The Role of the Apelinergic Axis in the Reproductive System Diseases

The presence of APLN/APJ has been demonstrated in the human ovary. APLN and APJ expression in granulosa cells and oocytes increases depending on follicle size and decreases in the luteal phase. It is suggested that APLN/APJ plays a role in the formation and regression of corpus luteum. Progesterone has been shown to stimulate APJ expression in granulosa cells (58, 59). Studies have shown that components of the apelinergic system are expressed in placental tissue in both normal pregnancies and those complicated by specific disorders (60). Lower serum APLN levels were detected in pregnant women (24-28 weeks) compared to non-pregnant groups (61). APLN also positively affects trophoblast survival by inhibiting the apoptosis of these cells (62). The production of placental hormones necessary for healthy pregnancy progression is related to the apelinergic system.

ELA is detected at the blastula and gastrula stages of earliest zebrafish development (63). Moreover, the expression and secretion of APLN/ELA changes during various stages of pregnancy. This may be related to changing endocrine functions (64). APLN reduces angiogenic activity during placental implantation and contributes to preeclampsia development (65). Additionally, the apelinergic system regulates glucose homeostasis in the fetus/newborn. Intravenous APLN administration increases placental glucose transport, while intraperitoneal adipokine injection in newborns increases glucose uptake in the lungs and muscles. During pregnancy, ELA also mostly takes part in glucose metabolism (64, 66).

9. The Role of the Apelinergic Axis in the Angiogenesis

Angiogenesis is the process of formation of new vessels from previously existing vessels. It is important in embryogenesis, such as tumor growth, myocardial infarction, and wound healing (67). The APLN system contributes to the physiological and pathophysiological regulation of blood vessel growth (68). Blood vessel structure is impaired in APLN knockout embryos(69). In a mouse model of vascular injury, APLN has been shown to promote vascular repair and endothelial cell differentiation (70). APLN may cause vasodilation or vasoconstriction, depending on the conditions. This dual effect of APLN is attributed to APJ receptors in endothelial and smooth muscle cells (71). There are also studies reporting that Elabela contributes to the regulation of angiogenesis (72). The APLN/APJ system seems promising in treating diseases related to endothelial dysfunction (73).

10. The Role of the Apelinergic Axis in the Diabetes and Pregnancy

Gestational diabetes and preeclampsia are significant problems during pregnancy. Preeclampsia affects the liver, kidney, and brain and is a cause of increased maternal/fetal morbidity and mortality (74). Although the etiology is not yet fully known, it is associated with antiangiogenic factors. The resulting uteroplacental ischemia causes proinflammatory cytokines to appear in the maternal circulation (75). Studies have found that APLN and APJ significantly regulate cardiovascular and fluid balance disrupted in preeclampsia (76). In samples from women with preeclampsia, it has been reported that APLN and APJ are increased in all placental compartments, with the most significant increase in villus endothelial cells (77). Circulating APLN was significantly increased in preeclampsia, indicating the role of APLN in the discrimination of early-onset preeclampsia (78). Different studies have found that serum APLN concentration is lower in patients with preeclampsia (79, 80). In another study, maternal serum levels of APLN-36 and APLN-13 were low in pregnant women with preeclampsia (81). Surprisingly, the placenta secretes ELA and may also participate in developing preeclampsia. Deleting the APELA gene in mice causes preeclampsia symptoms such as kidney damage, hypertension, and proteinuria (82, 83). According to the literature, APLN inhibits the development of the rat preeclampsia model, and administration of APLN to mice significantly reduces the negative symptoms of preeclampsia (84).

Gestational diabetes affects 14% of pregnancies worldwide, and physiological insulin resistance develops, facilitating the passage of nutrients

to the fetus. Slightly higher glucose supports fetal growth. However, this causes hyperglycemia and glucose intolerance in the mother (85, 86). Although the APLN/ELA system is thought to be related to the pathophysiological mechanisms of gestational diabetes, further research is needed to elucidate the mechanisms (83). One of the conditions that affects the fetus is intrauterine growth restriction. Intrauterine growth restriction can be defined as an infant's birth weight and length less than the 10th percentile at a particular gestational age (87). It has been reported that when normal pregnancies are compared with intrauterine growth restriction APLN serum level, it is 30% lower. ELA is downregulated in maternal serum compared to control, which shows a positive correlation with birth weight (88, 89).

Studies show that APLN concentration is increased in patients with type I and type II diabetes (90). APLN expression in adipocytes increases during adipogenesis and is regulated by insulin, growth hormone, and TNFα factors. There is a positive correlation between APLN plasma concentration and body mass index (91). Today, evidence shows that APLN is closely related to glucose metabolism and insulin sensitivity. The APLN/APJ system becomes a target for promoting glucose uptake, increasing overall glucose utilization, and improving insulin resistance, but dangerous effects are possible in the body due to abnormal APLN concentrations (10). APLN is predicted to have a regulatory role in energy metabolism and anti-obesity and anti-diabetic properties (92). The presence of maternal obesity is associated with gestational diabetes, preeclampsia, and cardiomyopathy (92). Again, the multifactorial pathogenesis of polycystic ovary syndrome is unclear, and studies report the role of adipokines in developing polycystic ovary syndrome (93). Hyperinsulinism and insulin resistance are common in women with polycystic ovary syndrome, and low-grade chronic inflammation is associated with an increased risk of early atherosclerosis and cardiovascular disease (94). There was a correlation between the concentration of APLN, glucose, and triglycerides in plasma in people with type II diabetes (95). Although it has been reported that APLN normalizes diabetes-induced renal hypertrophy and obesity-related cardiac hypertrophy, there is also evidence that it negatively supports retinal angiogenesis in diabetic retinopathy (96).

11. The Role of the Apelinergic Axis in the Endocrine System Diseases

The hypothalamus and pituitary gland are the primary sites of APLN action. It has been shown that APLN-13 application stimulates corticotropin-

releasing hormone and vasopressin release. This suggests that APLN may be necessary in regulating water intake in the endocrine axis (59). The APLN/APJ system is known to regulate the renin-angiotensin-aldosterone system, apoptosis, inflammation, and oxidative stress, which promote aging. Likewise, the APLN/APJ system contributes to anti-aging by regulating autophagy, stem cells, and the sirtuin family. The role of the APLN/APJ system in senescence stimulators, senescence inhibitors, and age-related diseases such as obesity, diabetes, and cardiovascular disease is also being investigated (97). It shows that the severity of depressive symptoms in elderly patients is positively related to plasma APLN levels. The higher the plasma APLN levels, the greater the seriousness of depression (98).

12. The Role of the Apelinergic Axis in the Locomotor System Diseases

Pathologically, the APLN/APJ system alleviates osteoporosis while aggravating the pathogenesis of osteoarthritis. Additionally, APLN expression can be regulated by continuous or intermittent exercise modes. Exercise-induced APLN may be a tool in the treatment of diseases and the regulation of physiological processes. Considering its pleiotropic effects on the locomotor system, the APLN/APJ system appears to be an important target in locomotor diseases (99). It has been reported that skeletal muscle atrophy caused by chronic kidney disease can be inhibited by exogenous APJ ligand supplementation and that the APLN-APJ system is effective in chronic kidney disease-induced skeletal muscle atrophy (100).

13. Conclusion

Although it has been seen in the literature that apelinergic agonists may effectively alleviate diseases or their complications in various disease models, further studies are needed to determine whether this axis can be an alternative strategy in the treatment (101).

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CHAPTER VII

THE EFFECTS OF DIABETES ON THE MALE REPRODUCTIVE SYSTEM

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1. Introduction

Diabetes (Diabetes mellitus, DM) is a chronic metabolic disease that causes chronic and acute complications due to increased blood glucose levels as a result of insulin deficiency and/or insulin resistance, which also significantly reduces the quality of life of the organism and can lead to death. This disease persists throughout life and requires constant monitoring and treatment (1-3). According to the International Diabetes Federation (IDF) report, while the number of diabetic patients worldwide is 537 million by 2021, it is predicted that this number will increase by 55% in 2030, reaching 643 million, and in 2045 it will reach 784 million. Aging, unhealthy nutrition, population growth, physical inactivity, and obesity are thought to be the reasons for this increase. According to IDF, there were 9 million diabetic patients (aged 20-79) in our country in 2021. Again, according to the IDF report, approximately 6.7 million people around the world died due to diabetes and complications caused by diabetes in 2021 (4). According to the 2019 report of the World Health Organization (WHO), diabetes ranked 9th among the causes of death in the world (5).

2. Diagnosis

According to the diagnostic criteria determined by the American Diabetes Association (ADA), the simplest diagnosis of diabetes is made when the fasting venous plasma glucose level is 126 mg/dL or higher than this value when

measured at least twice in a row. In addition, the diagnosis can be made when the venous plasma glucose level exceeds 200 mg/dL at any time during the day, regardless of fasting or satiety status, and the presence of symptoms such as polydipsia, polyuria, polyphagia, and weight loss. Also, the diagnosis is made by an oral glucose tolerance test (OGTT) performed after a fasting period of at least 8 hours, and the plasma glucose level at the 2nd hour is > 200 mg/dL (6).

3. Complications of Diabetes

Hyperglycemia, defined as a higher than normal level of sugar (glucose) in the blood, is the most important symptom of diabetes. High levels of glucose combine with proteins in the organism and turn into chemically recyclable glycosylation products. This conversion increases proportionately with the blood glucose level. Glycolization by glucose with collagen and other long-lived proteins in the walls of blood vessels or intersectal tissues turns into the end products of glycolization, which is impossible to recycle after a series of chemical reactions (7). Depending on this, diabetes causes acute and chronic complications. Acute complications include; diabetic ketoacidosis, hyperosmolar hyperglycemic state, lactic acidosis, and hypoglycemia. If hyperglycemia due to diabetes persists for a long time, chronic complications may occur. Chronic complications are of two types: macrovascular and microvascular complications. Macrovascular changes lead to the development of conditions such as coronary artery diseases, hypertension, stroke, dyslipidemia, and peripheral artery diseases (8). Macrovascular changes include diabetic neuropathy (damage to nerve fibers due to activation of glycosylation products and oxidative stress), nephropathy (kidney failure triggered by macrovascular complications starting with microalbuminemia) (9), and retinopathy (vision loss due to damage to small vessels in the retina of the eye and maculoedema). It can lead to various problems such as developing vision (10). In addition to these complications, diabetes can also cause important problems such as male impotence and infertility (9). Experimental and clinical studies have shown that these complications can be prevented or delayed if adequate metabolic control is achieved in type 1 and type 2 diabetes (11,12).

4. Classification of Diabetes

According to the American Diabetes Association 2020 diabetes classification, diabetes is divided into 4 main groups: type 1 diabetes, type 2 diabetes, gestational diabetes, and specific type diabetes due to other causes

(13). It is known that the majority of patients with diabetes consist of individuals with type 1 and type 2 diabetes (14).

4.1. Type 1 Diabetes

Type 1 diabetes is also called insulin-dependent diabetes or 'juvenile diabetes'. Type 1 diabetes mellitus (DM), which is common in the childhood age group, develops as a result of the destruction of the beta cells of the pancreas, which are involved in insulin production, due to ongoing autoimmune and non-autoimmune reasons. It is a chronic metabolic disease characterized by insulin deficiency (insulopenia) and hyperglycemia (15-17). Approximately 10% of diabetic patients have Type 1 diabetes, and it is known that the number of individuals with Type 1 diabetes is increasing nowadays (14). Although it can be seen in any age group, the most common age group is 7-15 years old (18). It is divided into two types, type 1a and type 1b, depending on the presence of autoimmunity. While immune-based Type 1a constitutes 90% of diabetic cases, Type 1b, which is also seen in the childhood age group and has negative autoimmune markers, constitutes 10% (19).

4.1.1. Etiology and Pathogenesis

Genetics, environment (diet, hygiene, toxins, and seasonal factors), and infections play important roles in etiology.

4.2. Type 2 Diabetes

Type 2 diabetes is also called non-insulin-dependent diabetes. Type 2 diabetes is a metabolic disease characterized by dysregulation of carbohydrate, lipid, and protein metabolism, with hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination of both. Type 2 diabetes accounts for more than 90% of all cases (20). It generally occurs between the ages of 30-50 and its incidence increases with age (21). The majority of patients (80-90%) have obesity.

Type 2 DM can be distinguished from Type 1 DM due to features such as its slower and more insidious course, obesity being a risk factor, lower levels of hyperglycemia, and generally the absence of ketoacidosis (22).

4.2.1. Type 2 Diabetes Risk Factors

Type 2 diabetes occurs as a result of the interaction of genetic, environmental, and metabolic factors. Risk factors for type 2 diabetes include body mass index

(BMI) >25, obesity, age (23,24), family history, physical inactivity, large waist circumference, high intake of trans fatty acids, diet with high glycemic index foods, psychosocial stress, depression, short sleep duration, smoking, low birth weight, gestational DM, genetic predisposition, and environmental irritant factors (such as chronic arsenic exposure) (25).

4.3. Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM) is defined as a type of glucose intolerance that develops in the second and third trimesters of pregnancy and causes hyperglycemia of variable severity (26). Gestational diabetes is a metabolic disease caused by the interaction between genetic and environmental risk factors. It is characterized by impaired pancreatic β-cell function as well as insulin resistance (27). Approximately 7% of all pregnancies result in GDM (28). Type 2 diabetes is diagnosed in 10% of women with a history of GDM soon after birth (27).

In pregnancies affected by gestational diabetes, the risk of cesarean and operative vaginal birth, increased risk of fetal malformation, macrosomia, shoulder dystocia, neonatal hypoglycemia, and hyperbilirubinemia are increased. Children affected by GDM also have a higher risk of developing obesity and T2DM early in life (29).

4.4. Other Types of Diabetes

Hyperglycemias that occur due to various reasons affecting the pancreas, such as genetic dysfunction in the β cells of the pancreas, drugs and toxic substances, exocrine pancreatic diseases, genetic syndromes, inflammatory diseases, and genetic disorders in the function of insulin are included in this group (30).

5. Infertility

Fertility in women is at its highest level in the twenties and thirties, while in men it is at a very high level until the forties (31). Infertility is defined as a disease of the male or female reproductive system in which pregnancy does not occur despite regular unprotected sexual intercourse for 1 year or more (32). In infertility, the male factor alone is 30%, the female factor alone is 40-50%, and the probability of a problem in both sexes is around 20%. Therefore, it is seen that men are directly or indirectly involved in the etiology of infertility at a rate of 50% (33). The fertility rate in men and women around the world is decreasing due to various factors, especially environmental changes (34).

The main causes of infertility have been identified as hormonal disorders, infections, diabetes, kidney failure, undescended testes, varicocele, genetic anomalies, chemotherapy, obesity, malnutrition, increased environmental pollution, exposure to various chemicals, radiation, cigarette consumption, use of alcohol, and addictive substances (35).

6. Effect of Diabetes on The Male Genital System

The effect of diabetes on the functions of the male genital system constitutes one of the most prominent complications in recent years. Subfertility and infertility cases are more common in diabetic male patients than in females (36,37). In two different studies, it is claimed that the prevalence of subfertility in diabetic men is 51%, and in another study in which 857 male patients were followed, the prevalence of infertility was 35% (38,39). Studies show that hypogonadism is quite popular in obese and diabetic men (40).

When the findings obtained from various studies were examined, it was observed that the decrease in fertility in diabetic men and animal diabetic models was accompanied by impaired spermatogenesis, degenerative and apoptotic changes in the testes, altered glucose metabolism in the Sertoli/blood-testis barrier, a decrease in testosterone synthesis, ejaculation dysfunction, and decreased libido (41).

6.1. Histological Changes Caused by Diabetes in The Testes

Diabetes causes changes in the functional and histological structure of the testis in men and animal diabetic models. Histological changes occurring in the testes include changes such as hypertrophy in the testicular stroma, atrophy in the seminiferous tubules, irregularity in the germinal epithelium, the appearance of giant cells and loss of germ cells, decrease in seminiferous tubule diameter and epithelial height, presence of germ cells at different development stages in the tubule lumen, vacuolization in Sertoli cells, cessation of spermatogenesis, increase in interstitial space volume due to edema and increase in connective tissue, an increase in the number of vacuoles and oil droplets in the Leydig cells and a decrease in their number, congestion in the interstitial area and thickening on the basement membrane of the tubulus (42-44). In addition, a decrease in the number of germ cells, clustering of epithelial cells, a decrease in stereocilium, and lipid vacuolization are observed in the epididymis (45,46), while wrinkled secretory epithelial cells, intraepithelial neoplasia, and enlarged secretory organelles occur in the prostate (47). In addition to the histological changes in the testis, a decrease in testicular and seminal vesicle weights and functional

disorders in Leydig cells were observed (48). It is thought that these changes are caused by oxidative stress and hormonal changes related to DM (49-51).

6.2. Neuroendocrine Regulation of Fertility in Diabetes

Functional and effective functioning of the Hypothalamic-Pituitary-Gonadal (HPG) axis is necessary for pubertal development, normal androgen production, and full reproductive capacity [52]. Gonadotropin realizing hormone (GnRH) secreted from the hypothalamus is an important hormone that controls gonadotropin production and testicular function (53). Metabolic cooperation between cells in testicular tissue is under hormonal control (50). Diabetes reduces the production of gonadotropin-releasing hormone (GnRH) by disrupting the hypothalamo-pituitary-gonadal axis, and decreases the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (50). Studies have shown that testosterone, FSH, and LH levels are low in men with diabetes (54). It has been determined that low LH levels impair spermatogenesis, and low FSH levels reduce the diameter of the seminiferous tubule and reduce the number of sperm (55). Shrinkage of Leydig cells and impairment of spermatogenesis were determined in brain-specific insulin receptor knockout mice (53,56). Once more, there is a clear connection between the levels of insulin and leptin. An essential hormone called leptin is released by fat cells and activates the brain to control fertility (41). It has been found that leptin levels decrease in patients with type 1 diabetes (57). In addition, germ cells in the seminiferous tubule epithelium are under both paracrine and endocrine control of Sertoli cells. In other words, hormonal fluctuations affecting Sertoli cells negatively affect germ cells, especially spermatozoa (50).

6.3. Decreased Sperm Quality in Diabetes

Type I diabetes can affect the expression of genes that lead to decreased sperm DNA repair, mitochondrial DNA deletions, and sperm motility (52). It has also been demonstrated that insulin levels affect the sperm acrosum and plasma membrane (48). In addition, studies have shown that hyperglycemia causes serious complications, especially in the hydrolysis of triglycerides, fatty acyl esters of cholesterol, and steroid hormones (58). While diabetes causes a decrease in sperm motility and number, it also causes an increase in the rate of abnormal spermatozoons (47,52,54,59). It has been determined that insulin treatment improves the defects occurring in spermatozoa (60).

6.4. Hypogonadism

Testosterone is a very important steroid hormone for the development of male reproductive tissues such as testis, and prostate and spermatogenesis (61). While it activates some genes that support spermatogonia differentiation in Sertoli cells, it also regulates the regular functioning of the HPG axis (62,63). Besides these, it affects energy metabolism and, accordingly, the oxidative balance (63). Male hypogonadism, also known as lack of testosterone production, is quite common in type 2 diabetes cases (64). This is due to damage to Leydig cell steroidogenesis by oxidative stress (49). Testosterone also has an important role in men's libido (sexual drive), erection and producing semen and spermatozoon. For this reason, it has been observed that diabetics cause low levels of testosterone, which in turn causes low libido, erectile dysfunction and a decrease in the increase of semen and spermatozoon (65-67).

6.5. Erectile Dysfunction

Erectile dysfunction (ED) is defined as the inability to achieve and maintain the penile erection necessary for sexual intercourse (68). Erectile dysfunction is a common condition in aging men, especially in those with diabetes (69). ED is seen approximately three times more frequently in diabetic men than in the normal population (70). Due to diabetes, increased advanced glycation end products (AGEs), increased levels of free oxygen radicals, decreased nitric oxide synthesis and its effector molecule cGMP, and erectile dysfunction due to neuropathic damage are observed (71).

6.6. Ejaculation Complications in Diabetes

Ejaculation complications occur as premature ejaculation, delayed ejaculation, and retrograde ejaculation (72). These complications are quite common in diabetic patients (73). Nerve damage to the penis in diabetes leads to ejaculation problems by causing decreased nerve sensitivity (74).

7. General Signaling Pathways Effective in Diabetic Infertility

Various signaling pathways are thought to negatively affect fertility due to hyperglycemia by diabetes. For example: oxidative stress, inflammation, apoptosis, angiogenesis, and autophagy.

7.1. Oxidative Stress

Diabetes is a metabolic disorder as well as a state of increased oxidative stress (75). In the case of hyperglycemia seen in experimentally diabetic rats

and diabetic patients, an excessive increase in reactive oxygen species (ROS) production and a decrease in antioxidant defense mechanisms cause oxidative stress (76-78). Oxidative stress plays an important role in the pathogenesis of diabetes. Increased free radicals in diabetes cause loss of membrane integrity in cells, structural changes in proteins, and genetic mutations by interacting with lipids, proteins, and nucleic acids (33).

Oxidative stress is shown to be the most important factor in the development of complications caused by diabetes such as, retinopathy, nephropathy, neuropathy, ischemic heart disease, and peripheral vasculopathy (75,79,80).

As a result of hyperglycemia resulting from diabetes, metabolic stress, hypoxia, and ischemic reperfusion resulting from changes in mitochondrial respiration, the auto-oxidation pathway, polyol pathway, hexosamine pathway, and non-enzymatic glycation end products cause tissue damage. These damages also increase ROS production (81,82). While high glucose levels cause cellular stress in the β cells in the islets of Langerhans in the pancreas, insufficient antioxidant enzyme activities include the beta cells in the pancreas to the tissues most sensitive to oxidative stress (83).

In studies conducted on experimental animals, streptozotocin (STZ), which has an N-nitroso-derived D-glucosamine structure, is thought to initiate diabetes by selectively damaging β cells in the islets of Langerhans in the pancreas through oxidative stress (84).

Antioxidant treatment in diabetes has been shown to increase the glycemic index, reduce diabetic complications, and have a protective effect against free radical-induced oxidative stress (3).

Sperm metabolism and DM are closely related to oxidative stress. The fertilization capacity of diabetic individuals is negatively affected by increased DNA damage in sperm resulting from high oxidative stress levels (85). Since the vascularization in the testis is weak, the amount of oxygen is low. Here, the low oxygen level protects the testis from free radical damage. Even though the amount of oxygen in the testis is low, the testis is very sensitive to oxidative stress due to the presence of unsaturated fatty acids and ROS-forming systems. Due to oxidative stress, both spermatogenesis and Leydig cell steroidogenesis in the testis can be damaged (49).

7.2. Apoptosis

Oxidative stress prevents cell division by affecting DNA replication. It also initiates a process that leads the cell to apoptosis (86). Germ cell apoptosis,

which occurs in mammalian testes under normal conditions, controls the excessive production of male gametes (87). It has been shown that there is a positive relationship between increased sperm damage and apoptosis, which is accompanied by ROS production (88, 89). Seminal plasma is equipped with antioxidants that function as free radical scavengers to protect sperm against oxidative stress. However, an increase in free radicals in the balance between free radicals and the antioxidant system may result in apoptosis (90). Therefore, it is known that DM affects sperm quality by causing damage to the nuclear and mitochondrial DNA of spermatozoa and, in addition, causes sexual problems such as impotence and decreased libido (50). In diabetic male patients, the percentage of spermatozoons that are immature, undergoing apoptosis, less motile, abnormal morphology is found to be quite high (91).

7.3. Inflammation

The male reproductive system suffers harm as a result of inflammation, one of the most prevalent influencing variables in diabetic circumstances (92, 93). Tumor necrosis factor-α (TNF-α), which can harm the testes and activate the inflammatory signaling pathway, and other pro-inflammatory substances are induced by hyperglycemia, according to studies (94, 95). Interleukin 1b, a pro-inflammatory cytokine, was found to be present at higher levels and interleukin 10, an anti-inflammatory cytokine, at lower levels in diabetic rats, according to Nna et al. (96).

TNF-α directly or indirectly suppresses spermatogenesis by inducing the production of molecules such as NO and ROS, damages sperm membranes, and reduces semen quality (94,97). One of the processes connected to the development of diabetes as a result of hyperglycemia is inflammation. Hyperglycemia presents itself as inflammation in the advanced stages of diabetes (98). Nitric oxide synthase (iNOS), one of the molecules produced in the inflammatory process, produces large amounts of toxic substances in testicular tissues or cells (92,99,100).

7.4. Angiogenesis

Angiogenesis is a physiological process in which new blood vessels form by budding from pre-existing vessels (101). By encouraging the growth of already-existing (angiogenesis) or new blood vessels (vasculogenesis), vascular endothelial growth factor (VEGF) significantly contributes to the development of new blood vessels. (101,102). VEGF also has an important regulatory role in

the testes by maintaining normal blood flow in the regional circulation (103). VEGF is abundantly produced by testicular Sertoli cells (104). Long et al. reported that testicular Sertoli cell viability and VEGF production decreased in diabetes; thus, this condition negatively affected testicular functions by causing dysfunction in the blood circulation (103). Other studies show that VEGF levels are lower than normal, along with increased apoptosis and testicular damage in the testes of diabetic rats. Therefore, it is thought that VEGF reduction may play a role in male dysfunction in diabetes (105,106,107).

7.5. Autophagy

Autophagy is a mass degradation process involved in the clearance of damaged proteins and organelles (108). Traditionally, autophagy has been considered a survival mechanism for cell quality control in response to cellular stress. (109). If the cell cannot obtain the necessary nutrients from its environment, it continues its existence by eating itself from within. Autophagy can be triggered by cellular stress such as pathogen infection, hypoxia, nutrient depletion, or reactive oxygen species (ROS). When its control is impaired, it causes cancer, early dementia, some hereditary diseases, Alzheimer's, and infections (110). Therefore, autophagy plays a role in the pathogenesis of many diseases.

Autophagy-associated proteins such as Beclin-1, light chain 3B (LC3B), and ubiquitin-binding protein (p62/SQSTM1) are markers of autophagy (111). A study showed that insulin deficiency (type 1 diabetes) or insulin resistance (type 2 diabetes) increases autophagy activity (112). Increased autophagy has been shown to lead to the degeneration of germ cells. Because of this, increased autophagy can damage mitochondria and the endoplasmic reticulum, which compromises the testes' integrity. It may also affect testicular growth and the development of normal physiological functions (113).

8. Conclusion

Studies on fertility show that the increase in the incidence of DM is closely related to the decrease in fertility (3,86). Recent studies show a continuing increase in the number of young patients with type 1 and type 2 diabetes. The fact that this increase affects many men with diabetes in active reproductive age can be considered an indication that fertility problems due to DM will increase in the near future.

It is also known that hormonal and metabolic changes resulting from oxidative stress due to DM play a key role in the development of male infertility.

Glucose homeostasis is very important for spermatogenesis and maintaining the fertilization capacity of spermatozoons. Therefore, studies suggest that the complications caused by DM in the testicles are related to the absence of insulin, the leading hormone in glucose homeostasis.

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CHAPTER VIII

INVESTIGATION OF THE CHEMICAL COMPOSITION, ANTIOXIDANT, AND ANTIPARASITIC EFFECTS OF *ROSMARINUS OFFICINALIS* L.

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1. Introduction

Throughout the history of humanity, people have endeavored to identify and understand the plants in their environment, determining which ones are safe to eat and which are not. Plants used for shelter, protection, and nourishment have also been employed, especially after the transition to settled life, for the purpose of treating illnesses. Archaeological excavations, such as those conducted at Shanidar Cave in Northern Iraq, have provided evidence of the use of certain plants by a shaman for their afterlife, as indicated by the results of these studies. In the course of these excavations, pollen from various plant species, including yarrow, rose mallow, canary grass, purple crocus, marigold, and mallow, was discovered in the burial site of the shaman. (1)

In recent years, the utilization of plants for the prevention and treatment of diseases has significantly increased worldwide due to various factors. The rosemary plant, known as *Rosmarinus officinalis*, is a fragrant, evergreen, needle-like-leaved, woody, perennial plant native to the Mediterranean region. This plant belongs to the species *Rosmarinus* within the Lamiaceae family. (2)

Throughout the development of medicinal and aromatic plants, they accumulate and store a wide variety of active compounds. The quantities of these active substances vary depending on factors such as the plant's species, geographic location, harvesting and drying conditions, and post-drying preservation methods. (3) Many studies have been conducted to understand the health benefits of these medicinal plants and how they can be more beneficial. Particularly noteworthy are the studies related to bioactive compounds found in foods. Research on the positive effects of the rosemary plant, which contains a significant amount of antioxidant compounds, on human health has been gaining importance in many countries around the world. (1)

The primary objective of this study is to investigate the chemical composition, antioxidant properties, and antiparasitic effects of *Rosmarinus officinalis* L., a plant native to our country and commonly known as 'Kuşdili' among the public.

2. *Rosmarinus officinalis* L.

Rosemary (*Rosmarinus officinalis* L.) is a highly consumed aromatic and medicinal plant from the Lamiaceae family. Its dried or fresh leaves are commonly used in traditional cuisine and folk medicine.

Rosmarinus officinalis L. can be utilized in various ways. It can be consumed fresh immediately after harvesting, dried for later use, or steeped in water for herbal preparations. (4)

The flower parts of the rosemary plant can be boiled in water to make a stimulating syrup. Its leaves are also used to extract oil, which is utilized in the production of perfumes and cleaning products. Additionally, due to its appealing appearance, this plant is used for decorative purposes in gardens. *Rosmarinus officinalis* L. is native to the Mediterranean basin. It is cultivated in various regions around the world, including Madeira Island in Portugal, the Canary Islands in Spain, North African countries such as Algeria, Libya, Morocco, and Tunisia, as well as in Western Asian regions including Cyprus, Yugoslavia, Greece, Italy, France, South Africa, India, China, Australia, England, and the United States. (5)

In Turkey, rosemary (*Rosmarinus officinalis* L.) is most commonly found in the Adana, Hatay, Mersin, and Çanakkale regions. (6)

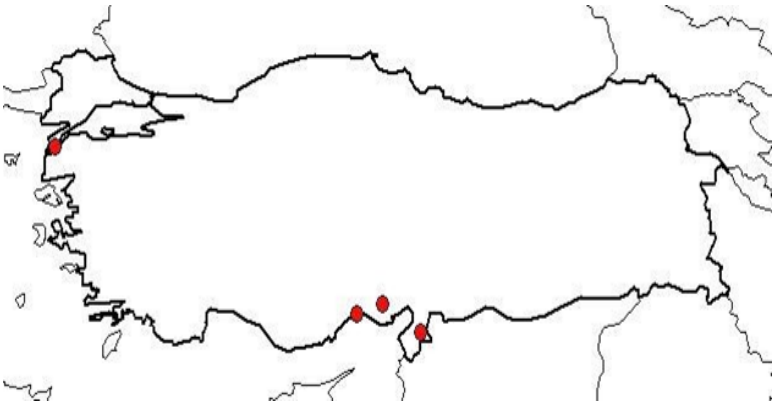

Figure 1. Distribution of *Rosmarinus o icinalis* L. Plant in Turkey. (6)

The family in which rosemary (*Rosmarinus officinalis* L.) is categorized, known as Lamiaceae, is also referred to as the "Mint family" in English and "Ballıbabagiller" in Turkish. This family includes a total of 236 genera and 7280 plant species, including herbs such as basil, thyme, mint, and lavender. When looking at the regions where Lamiaceae family plants are cultivated, they tend to yield the best results in temperate climates. (7)

2.1. Anatomy and Morphology of Rosmarinus officinalis L.

Its flowers are small and grouped at the ends of branches and leaf axils. It is in bloom all year round. The flowers are arranged in a raceme shape along an axis. The sepals are tubular, bilabiate, and densely hairy. The petals are also tubular and bilabiate. The flowers come in shades of bluish-white, purple, and lilac. There are two narrow lobes on the upper lip, and three narrow lobes on the lower lip. The middle lobe of the lower lip is larger and concave compared to the others. There are two stamens. The filament is longer and curved compared to the corolla tube, it is purple, and it has a small tooth-like projection at its base. The female organ consists of two carpels, the style is long and curved, and the stigma is bifid. Nectaries are present in the flowers. It is an evergreen shrub with dense branching that can grow tall. The branches are generally upright and 50-100 cm in length. The leaves are 10-25 x 1-2 (-4) mm, leathery, narrow, linear, with edges curved downward, dark green on the upper surface, long and softly hairy,

and light-colored on the lower surface, densely soft hairy. The flower stems are densely hairy and shaped like white stars. The calyx is 3-4.5 mm, divided into two, white and densely hairy, gradually becoming veined and somewhat bare as the fruit grows. The corolla is light blue and bilabiate. (5)

The leaves of R. officinalis L. are covered with a thick cuticle. There is a single-layered epidermis on both the adaxial and abaxial surfaces. The leaves bear non-glandular trichomes on both sides of the lamina but do not have glandular trichomes. Non-glandular trichomes are found on the veins and leaf margins. Some non-glandular trichomes are unbranched, while others are multicellular and branched. The fruits are fleshy and hard in structure. (8)

2.2. Chemical Composition of Rosmarinus officinalis L.

The most abundant primary components of the rosemary plant include carnosic acid, carnosol, rosmarinic acid, and hesperidin. Among the most effective antioxidant components are cyclic diterpenes, difenols, and carnosolic acid. Additionally, it contains carnosic acid, epirosmanol, rosmanol, methyl carnosate, and isorosmanol (8). The essential oil of rosemary plant contains 1,8-cineole (46.4%), camphor (11.4%), and α-pinene (11.0%). In addition, it also contains various caffeic acid derivatives. These compounds react with available metal ions, forming chelates; as a result, they interact with peroxide radicals and thus stabilize these free radicals. (9)

On the other hand, the bioactivities of rosemary extracts include properties such as anti-inflammatory, antidiabetic, hepatoprotective, and antimicrobial activity. These bioactivities are associated with phenolic compound components, primarily caffeic acid, rosmarinic acid, and carnosic acid. (10)

The physiological properties of rosemary essential oil have been published by the Research Institute for Fragrance Materials (RIFM). As known, rosemary possesses strong antioxidant properties. It is known that the carnosic acid in its leaves has a much stronger antioxidant effect than butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which have antioxidant effects in soybeans. According to this research, rosemary extracts prevent the formation of skin tumors in test animals and, in particular, its oil reduces insulin secretion by inducing hyperglycemia. Besides the essential oil, the main component is rosmarinic acid. Different chemical compounds are present in various parts of the plant. These can be classified as vitamins, minerals, fatty acids, nitrogenous compounds, organic acids, monosaccharides, and phenolic compounds. (11)

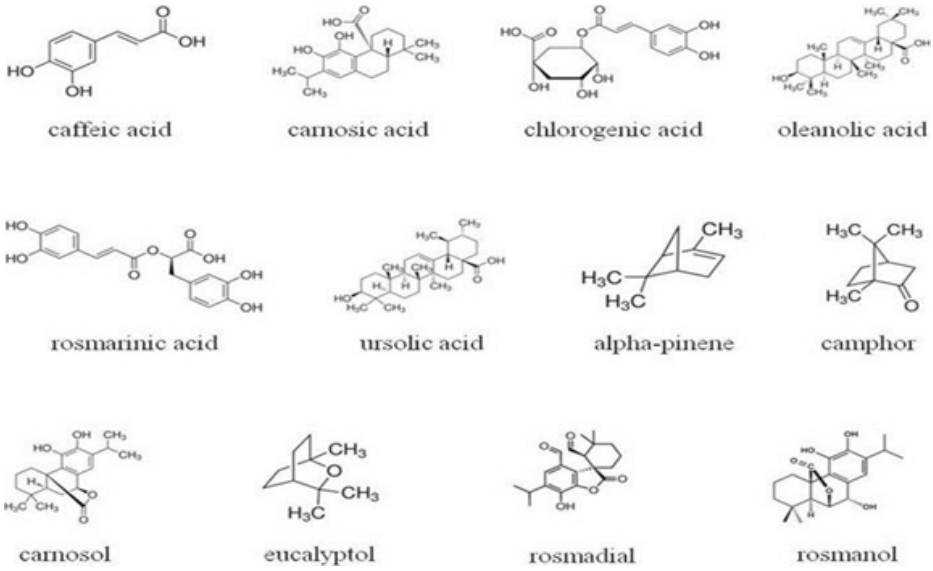

Figure 2. Chemical Structures of Some Secondary Metabolites of *Rosmarinus Officinalis* L. Species (11)

2.2.1. Rosmarinic Acid: A Potent Antioxidant Compound and Its Chemical Structure

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. It is commonly found in species of the Boraginaceae family and in the Nepetoideae subfamily of the Lamiaceae family. However, it is also found in species from other high plant families and in some fern species. It possesses a range of biological activities, including antiviral, antibacterial, anti-inflammatory, and antioxidant properties. The presence of rosmarinic acid in medicinal herbs and spices is associated with beneficial and health-enhancing effects. In plants, it is expected to act as a preformed structurally accumulated defense compound. (12)

Rosmarinic acid (RA) was first isolated from *Rosmarinus officinalis* L. in 1958 by Scarpati and Oriente. It is widespread in a wide range of plants and serves as a bioactive component in various medicinal plant species. It is a naturally occurring product commonly found in the plant kingdom. The compound is synthesized from L-phenylalanine and L-tyrosine amino acids in the Lamiaceae family. All the enzymes of the biosynthetic pathway and a few genes are known. (12)

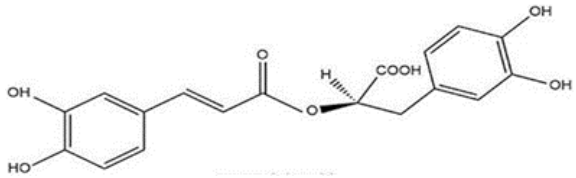

Figure 3. Chemical Structure of Rosmarinic Acid. (13)

2.3. Antioxidant Effects of Rosmarinus officinalis and Studies on This Effect

Rosmarinus officinalis L. is a significant antioxidant due to its polyphenol content. Consequently, numerous studies have been conducted on it, and it has been considered as a potential anticancer drug. It demonstrates this antioxidant effect through its capacity to act on free radicals.(14)

The composition of rosemary extract contains many compounds with high antioxidant power. These compounds primarily belong to the classes of phenolic acids, flavonoids, diterpenoids (carnosol and carnosic acid), and triterpenes. Among these compounds, rosmarinic and carnosic acids have the most significant impact on rosemary's antioxidant activity. (15)

In recent times, there have been many studies regarding the protective role of plants rich in phenolic compounds in food. The protective role of the *Rosmarinus officinalis* plant has been approved by EFSA (European Food Safety Authority) with the code E392. (16)

When examining studies on the antioxidant-effective active ingredients of *Rosmarinus officinalis* L., it has been observed that the antioxidant effect of these active ingredients varies depending on various environmental factors. (17)

In a study conducted in 2001, which examined antioxidant-effective substances such as *Aloe vera*, rosemary, and tea catechins, it was observed that, unlike other substances, tea catechins and rosemary had a stronger antioxidant effect. (18)

In another study, the antioxidant effect of the active ingredients of certain plants such as rosemary, broccoli sprouts, and citrus fruits on sunflower oil was investigated. When examining the effects of these plants, it was observed that sunflower oil, which contains a high amount of oleic acid, was protected against lipid oxidation. (19) In a study conducted in 2010, sheep were given varying amounts of rosemary, and the results showed that lipid oxidation was indeed inhibited. (20)

In a different study, the phenolic compounds contained in dill, rosemary, and fennel plants and their resulting antioxidant effects were examined. The study found that rosemary had a total phenolic compound content of 3367.24 mg GAE/100g, and it was determined to be the most effective plant in terms of antioxidant activity. (21)

In a study conducted in 2017, rosemary plants were ground and applied to the surface of some trout due to their natural antioxidant properties. The trout with rosemary extract applied to their surface and those without were packaged and cooked after allowing air exposure. After cooking, they were stored until they deteriorated at approximately 4 degrees Celsius. During storage, they were evaluated every 5 days based on their odor to understand spoilage. It was observed that the trout without rosemary extract deteriorated on the 40th day, while those with the extract deteriorated on the 45th day. This indicates that rosemary extended the shelf life of packaged foods by an average of 5 days and showed no negative aspects of its use in food. (22)

2.4. Rosmarinus officinalis L. Species and Some Types of Cancer It Is Effective Against

2.4.1. Colon Cancer

Colon and rectum cancer types are the third most common cancer types in the world. According to the numerical data released by the World Health Organization in 2018, there were 1.8 million new cases of colon cancer and 862,000 deaths from this cancer. The development of this cancer is a multi-step process involving successive gene loss. Diagnosis is only possible when the patient shows symptoms or as a result of a screening program. (23, 24)

When it comes to the treatment of colon cancer with rosemary, the most dangerous cells in colon cancer are HT-29, HCT116, and SW480 cells. Rosemary extract has significantly reduced the survival of these cells. Additionally, it was observed that it stimulated programmed cell death in HCT116 cells. (25)

Various components found in plant-based foods, medicinal herbs, and their bioactive compounds have demonstrated protective effects against various types of cancers, including colon cancer.(26) In a study conducted in 2019, the anticancer effect of the *Rosmarinus officinalis* plant extract (RE) was investigated. When examining modifications to the cell cycle of colon cancer cells, a decrease in the G0/G1 phase was observed, while an increase in cancer cells in the G2/M phase was noted. It was observed that RE strongly inhibited

the proliferation, migration, and consequently, colony formation of colon cancer cells. (27)

In another study that used colon cancer cell lines, it was again demonstrated that the extract of *Rosmarinus officinalis* L. plant exhibited anticancer properties. The colon cancer cell line Caco-2 was exposed to this extract, and it significantly reduced colony formation after 24 hours. Similarly, when the SW480 colon cancer cell line was exposed to this extract, it was observed that the extract inhibited cell growth within 48 hours and induced cell cycle arrest. Additionally, *Rosmarinus officinalis* was found to increase the proliferation of 5-fluorouracil (5-FU), a chemotherapeutic drug. All of these studies have shown that *Rosmarinus officinalis* L. plant extract, at concentrations between 20-100 µg/mL, can consistently inhibit cell growth and viability, potentially playing a role as an anticancer agent in colon cancer cells. (28)

2.4.2. Pancreatic Cancer

Pancreatic cancer is more common in men than in women. It is more prevalent in the black population compared to whites and is also more common in urban areas than in rural areas. While there are several factors that increase the risk of this cancer, such as coffee consumption, alcohol consumption, and pancreatitis, the only proven risk factor is smoking. (29) Advances in molecular biology have greatly improved the understanding of the pathogenesis of pancreatic cancer. Despite the resistance of pancreatic cancer to current treatments, new methods are being researched. (30)

In a study conducted in 2014, the anticancer effects of rosemary extracts (RE) on pancreatic and colon cancer cells were evaluated. It was observed that cancer cells sensitive to rosemary extract showed a decrease. In terms of possible mechanisms, an increase in the metabolic-related gene GCNT3 and a decrease in the potential epigenetic modulator miR-15b were associated with the anticancer effect of rosemary. (31)

2.4.3. Breast Cancer

Breast cancer is a highly prevalent global malignancy and a leading cause of cancer-related deaths. Breast cancer is classified into three subtypes based on the sensitivity of tumors to chemotherapeutic agents. The first subtype is estrogen receptor-positive (ER+), responding to estrogens; the second is human epidermal growth factor receptor 2 (HER2) overexpressed and can be ER+ or ER−, and the third subtype (iii) is the triple-negative subtype (TN), which

lacks ERα, progesterone receptor, and HER2 expression. Rosemary extract was investigated for its antioxidant effect by applying it to all three subtypes of breast cancer (ER+, HER2, TN) at concentrations of 1-120 µg/mL for 48 hours, and rosemary extract caused dose-dependent inhibition of cell viability in all subtypes of breast cancer cells. Furthermore, it was observed that it enhanced the efficacy of the monoclonal antibody (mAb) trastuzumab and the chemotherapeutic drugs tamoxifen and paclitaxel used in breast cancer treatment. (32,33,34)

2.4.4. Skin Cancer

Skin cancer is responsible for most malignancies worldwide. Skin cancer is divided into melanoma and non-melanoma skin malignancies. Non-melanoma skin cancer includes basal cell carcinoma and squamous cell carcinoma. The most significant risk factors are fair skin and chronic exposure to ultraviolet B. Primary prevention for this type of cancer is achieved by avoiding sun exposure and tanning. (35)

In a study published in the Cancer Research journal in 1994, conducted at the Cancer Research Laboratory of Rutgers University School of Pharmacy in New Jersey, experimental mice were exposed to cancer-inducing chemicals applied in two stages to create skin cancer in the animals. The mice were then divided into groups. One group had rosemary extract regularly applied to their skin, while the other two groups had rosemary's active compounds, carnosol and ursolic acid, applied. The control group received no supplements. It was found that the development of skin cancer was significantly lower in the groups where rosemary extract and active compounds were applied compared to the group that received nothing. The rate of detection in the group where rosemary extract was applied decreased from 64% to 99%. For those in the ursolic acid group, this rate was 61%, and for those in the carnosol-only group, protection was observed up to 78%. In another study, the clinical effects of rosemary on mouse skin cancers initiated with DMBA and promoted with croton oil were analyzed. The study examined parameters such as action duration, cancer formation and size, along with oxidative stress assessments. The findings indicated that rosemary extract might prolong the duration of action and reduce cancer formation, cancer burden, and cancer size. (36)

2.4.5. Liver Cancer

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and ranks as the third leading cause of cancer-related deaths. In a study published

in 2008, the expression of the bcl-2 and bax genes in the HepG2 liver cancer cell line was investigated after apoptosis induced by essential oils obtained from *Rosmarinus officinalis* through steam distillation. The findings revealed that the expression of the bcl-2 gene decreased while the expression of the bax gene increased, depending on both dosage and time. Consequently, it was understood that essential oils extracted from *Rosmarinus officinalis* could influence the expression model of bcl-2 and bax genes, leading to an enhancement of apoptosis in the HepG2 liver cancer cell line. (37,38)

2.4.6. Lung Cancer

In 2018, the American Cancer Society reported approximately 234,030 new cases of lung cancer and 154,050 deaths from the disease in the United States (56). In a study conducted in 2016, the effect of rosemary extract on the growth of human lung cancer cells and programmed cell death, as well as its impact on signaling events, was investigated. The results of this study showed that rosemary extract inhibited cell growth, reducing the survival of cancer cells and increasing programmed cell death. (39)

2.4.7. Blood Cancer

A study was conducted with rosemary extract on leukemia cell lines, HL-60 and K-562, as well as on the murine RAW264.7 macrophage/monocyte cell line. As a result, a significant decrease was observed in HL-60 and K-562 cells. Additionally, cell survival was reduced, and programmed cell death increased in K-562 cancer cells. Rosemary extract also enhanced TNF-alpha-induced apoptosis in U937 cells, another type of leukemia cell, while reducing ROS production. (40)

2.5. Antiparasitic Effect of Rosmarinus officinalis L. Species

Today, tropical diseases pose a significant risk to the global human and animal populations. These diseases are often overlooked and belong to a challenging group of illnesses to control due to factors such as high drug costs, drug resistance, and limited access to existing medications, especially in developing countries. Natural products continue to be an important source of raw materials for the development of new therapeutic agents. In a study, the antitrypanosomal properties of the chloroform extract of *Rosmarinus officinalis* were investigated. This study utilized a total of thirty rabbits, approximately 5-6 months old and weighing 1.5-2.5 kg each. The progression of *Trypanosoma evansi* infection in the rabbits was monitored for 48 days

following the infection. Blood samples were collected to measure hematological and biochemical parameters. The results showed abnormalities in all examined parameters in the infected group. In contrast, rabbits treated with the chloroform extract of *Rosmarinus officinalis* exhibited normalization of hematological and biochemical values, supporting the antiparasitic properties of this plant species. (41)

In a different study, the antifungal and antiparasitic effects of *R. officinalis* species were examined. This study investigated the effects of the species' methanol extract on the parasite *Trichomonas vaginalis*. This parasite is a sexually transmitted flagellated protozoan species. (42) In women, it commonly causes vaginal and urethral infections but can also lead to infections in the cervix, Bartholin's glands, or bladder. In men, this parasite is usually found at the tip of the urethra but can rarely affect the prostate gland, seminal vesicles, and epididymis. It can cause various diseases, such as vaginitis, urethritis, and prostatitis. According to the results of this study, the parasite was observed to become inactive within 4 hours at a concentration of 0.0001 and within 2 hours at a concentration of 0.0002. (43)

Rosmarinus officinalis (rosemary) has been studied for its effects on different parasites through in vitro and/or in vivo tests. For example, based on the results of epimastigot inviability measurements on *Trypanosoma cruzi*, the essential oil of *Rosmarinus officinalis* has been found to be effective at concentrations of 50 and 100 µl/mL. (44) On *Acanthamoeba polyphaga*, essential oil concentrations of 1, 2, and 4 mg/mL were investigated, and cell death was recorded as 100% after 144 hours at 2 mg/mL and 4 mg/mL concentrations. At a concentration of 1 mg/mL, cell death was recorded as 86% after 144 hours. Additionally, changes in trophozoite morphology were observed. (45)

Leishmania major infection and its effects on *Rosmarinus officinalis* essential oil and nanoemulsion concentrations were evaluated in a different study. According to the results, at a concentration of 0.125 µg/mL for *Rosmarinus officinalis* essential oil, the macrophage infection rate was found to be an average of 39.33% ± 6.02%. When evaluating the effect of this extract on *Leishmania major* infection using a concentration of 0.0625 µg/mL with nanoemulsion, the macrophage infection rate was found to be an average of 54.33% ± 7.02%. These results indicate that both the essential oil and nanoemulsion of *Rosmarinus officinalis* can affect *Leishmania major* infection at specific concentrations, reducing the infection rate in macrophages. (46)

Additionally, the essential oil of *Rosmarinus officinalis* has been studied at different concentrations for its effects on parasites such as *Toxocara* spp.,

Haemonchus spp., and *Echinococcus granulosus*, and its impact has been observed. (47, 48)

3. Conclusion

This study examined research on the phenolic compounds, antioxidant, and antiparasitic effects of Rosemary (*Rosmarinus officinalis* L.) plant. According to these studies, it can be stated that the species exhibits significant antioxidant and antiparasitic activity. Medicinal and aromatic plants with a long history of use have found applications in the treatment and prevention of various diseases today, primarily due to their metabolites. Among these aromatic plants, the secondary metabolites involved in the therapeutic effects of *Rosmarinus officinalis* L. have been the focus of many studies.

Rosmarinus officinalis is commonly used in traditional medicine due to its effects such as fever reduction, antispasmodic properties, and promoting sweating. Extracts and essential oils of this species are not only used for lowering blood pressure and increasing menstrual flow but also find widespread use in cosmetics. The use of rosemary extract in cosmetics is quite common, and preparations containing it have been shown to promote hair growth and be effective in preventing scabies. *Rosmarinus officinalis* is one of the oldest medicinal plants used centuries ago to enhance memory and boost brain activity. (43)

However, there are important considerations to be aware of when using the essential oil and extracts of *Rosmarinus officinalis*. Rosemary is contraindicated in cases of significant skin wounds, severe fever and infections, individuals with heart failure, and during pregnancy. In the past, instances of using rosemary outside of therapeutic purposes and in high doses have resulted in cases such as inducing miscarriages, spasms, vomiting, kidney damage, and even death. It should be especially noted that caution should be exercised in the use of rosemary, particularly in pregnant women and children under the age of 12. Products derived from *Rosmarinus officinalis* L., when used orally or topically, are generally well-tolerated. Excessive oral consumption may lead to stomach and intestinal irritation, cramps, and, in advanced stages, kidney damage. (49)

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