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Test animal models and measurement parameters in ischemic stroke

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Abstract

Background: Ischemic stroke is an acute neurological injury resulting from focal damage to the central nervous system due to vascular obstruction and subsequent decrease in cerebral blood flow. Numerous animal models of ischemic stroke have been established to investigate its mechanism, pathophysiology, and risk factors. The animal model of ischemic stroke includes a global ischemia model and a focal ischemia model. This article describes various parameters, including hematological, biochemical, cytological, histological, and molecular factors, along with diverse biomarkers, that may support research in the development of novel, safer, and more effective therapeutic agents for ischemic stroke using animal models.

Objective: This research seeks to determine the appropriate test animal model and parameters for ischemic stroke experiments.

Method: A complete literature search was conducted across multiple databases, including NCBI, PubMed, and additional sources.

Results: According to reference studies, the animal model test in the ischemic stroke experiment comprised a focal ischemia model and a global ischemic model. The focal ischemic model is more pertinent to ischemic stroke in humans compared to the global ischemic model. In addition, focal ischemic models, including Middle Cerebral Artery Occlusion (MCAO), have been utilized in over 40% of 2,582 nerve protection trials. The wide variety of test animal models possesses distinct advantages and disadvantages, making it crucial to select the appropriate model. The parameters of ischemic stroke, including hematology, biochemistry, cytology, histology, and molecular analysis, together with their biomarkers, can help in identifying the incidence of ischemic stroke in test animals.

Conclusion: The focused ischemia model is a more pertinent animal model for ischemic stroke in relation to humans than the global ischemic model. Parameters utilized for the identification of ischemic stroke encompass hematology, biochemistry, cytology, histology, and molecular biology.

Keywords: Global ischemic, focal ischemic, parameters, ischemic stroke

1. Introduction

Ischemic stroke is the most frequent case, which is about 80% of all stroke cases (Donkor, 2018). Ischemic stroke is an acute neurological condition that occurs when blood vessels to the brain become blocked, so that it damages brain tissue due to lack of oxygen and blood (Bansal *et al*., 2013). Ischemic stroke is generally caused by a blockage of brain blood vessels due to atherothrombosis that forms in the carotid artery or other blood vessels leading to the brain. This atherothrombosis can reduce or stop blood flow to the brain, which is supplied by the internal carotid artery and the branching vertebral artery of the heart aorta (Yueniwati, 2015). To understand more deeply about the various mechanisms of ischemic stroke, Animal modeling is a trustworthy and useful technique.

In recent years, the use of animal models has contributed to an in-depth understanding of the pathophysiological mechanisms of ischemic stroke. Ischemic stroke has been studied in

a variety of animal species. Animal modelling for stroke is now evolving rapidly with the emergence of new models and approaches that are more relevant and accurate, allowing for more effective research. The ischemic stroke model consists of global ischemic and focal ischemic. Focal ischemic stroke models are more applicable to human ischemia they are most frequently utilized in human ischemic stroke simulations. Examples of these models include thromboembolic models and Middle Cerebral Artery Occlusion (MCAO) (Li & Zhang, 2021). However, each model has its own advantages and disadvantages, which is why selecting the right model is crucial to ensuring that it is valid and dependable for ischemic stroke testing.

In addition to considering the selection of methods in the animal model of ischemic stroke, it is also necessary to understand several parameters or biomarkers used in ischemic stroke testing. Parameters are components that can help in identifying the occurrence of ischemic stroke. Some of the parameters discussed in this journal are hematology, biochemistry, cytology, hispatology and molecular parameters. Biomarkers are biological indicators that can be found in blood, body fluids or tissues that are used to detect disease conditions, predict risks and monitor response to treatment (Kim *et al*., 2013).

The review reviewed here provides an overview of the currently available ischemic stroke models and explains their advantages and disadvantages in selecting a suitable model. In addition, it shows a comprehensive analysis of various types of stroke parameters and biomarkers that can improve understanding in ischemic stroke testing so that it can support research in the development of new therapeutic agents.

2. Method

The method of writing of this review article involved literacy research from various peer-reviewed journal articles from NCBI, PubMeb, and other online journal sources that discuss parameters and biomarkers of ischemic stroke testing and animal models of ischemic stroke. The search keywords used are *animal model of ischemic stroke, global ischemic stroke, focal ischemic stroke, Two-Vessel Occlusion* (2-VO), *Four-Vessel Occlusion (4-VO), Model oklusi aorta/vena cava dan cardiac arrest, Chemical and gas hypoxia, Intraluminal MCAO (Middle Cerebral Artery Occlusion), Endothelin-1 induced stroke (ET-1), Electrocoagulation model of MCAO, Cerebral photothrombosis, Cerebral thromboembolism, hematology biomarker of ischemic stroke, molecular biomarker of ischemic stroke, histology biomarker of ischemic stroke, biochemical biomarker of ischemic stroke, sitology biomarker of ischemic stroke.*

The inclusion criteria in this article review include animal models and parameters and biomarkers used in ischemic stroke testing. Exclusion criteria include articles published under 2012.

3. Results and discussion

3.1. Animal model of ischemic stroke

Some of these animal models are designed to address specific risk factors. Animal models of ischemic stroke are divided into global ischemic and focal ischemic. In the global ischemic model, Ischemic is caused by a drastic decrease in blood flow so that it has an impact on metabolism and nerve function. In the focal ischemic model, blockage of blood flow occurs in an area of the brain called the ischemic nucleus (Kumar *et al*., 2016).

3.1.1 Global ischemic model

The global ischemic model is a relevant model for brain damage due to cardiac arrest. However, the focal ischemic model is more relevant to ischemia in humans (Li & Zhang, 2021). Here are some of the methods in the global ischemic model.

a) Two-vessel occlusion (2-VO)

The 2-VO is a model performed by inducing ischemia in the frontal brain, by combining bilateral communist carotid artery occlusion and systemic hypotension (Kumar *et al*., 2016). This model can induce hippocampal injury, resulting in memory impairment and cognitive deficiencies (Xu *et al*., 2016; Yabuki *et al*., 2015). According to Chen *et al* (2019), this 2-VO is suitable for studying the immune response and nerve recovery process after cerebral ischemial. In addition, according to Pontarelli *et al* (2012), this model provides optimal results compared to the 4-VO model. However, in the study of Bayat & Haghani (2017), it was stated that 2-VO can reduce brain blood flow in mice. Consequently, 2-VO would not be a suitable technique for causing acute ischemia in rats.

The advantages of this model are that it is simple in the surgical process and easy to reperfute. This model's low reproducibility and potential for hypotension are prominent drawbacks. Compared to the Four-Vessel Occlusion (4-VO) model, this model has additional benefits (**Table 1**).

b) Four-vessel occlusion (4-VO)

The 4-VO model involves permanent occlusion of the vertebral artery using electrocoagulation on the first day, which is then followed by temporary (reversible) occlusion

of the communist carotid artery on the second day (Lu *et al*., 2016). The advantages of this model are that it is easy to prepare, the rate of ischemic nerve damage is predictable, and the incidence of seizures is low. The main limitations of this model are the long-term two-stage surgical procedure and the risk of permanent occlusion of the vertebral artery (Table 1) (Li & Zhang, 2021).

c) Cardiac arrest

The most common cause of ischemic stroke in humans is usually caused by cardiac arrest. Intracardiac KCl injections or other cardiolegic medications can cause this condition. In addition, cardiac arrest can also be induced surgically by pressing large blood vessels in the mediastinum against the inner wall of the chest cavity using a hook (Kumar *et al*., 2016).

This model is a commonly used method to create CGBI (Complete Global Brain Ischemia) and can be used in neuroprotective drug research. However, systemic blood flow disorders not only cause damage to the brain but also have the potential to trigger various complications outside the central nervous system. In addition, the complexity of the procedure, the low survival rate, and the high risk of coma are the main challenges in this model. Therefore, intensive care after ischemic is urgently needed, especially in the early days (Table 1) (Dave *et al*., 2013).

d) Chemical and gas hypoxia

This model has the potential to cause obstructions in the blood vessels of the brain, leading to disruption of oxygen and nutrient supplies. Given that the brain is highly sensitive to hypoxia conditions, damage to brain tissue can occur within five minutes after oxygen flow has stopped. After ten minutes of exposure to gas-induced hypoxia, the optic tectum usually shows signs of infarction (Yang *et al*., 2021). This approach has benefits when it comes to screening drugs for stroke treatment and is relatively easy to use. However, this method was only able to model the mechanism of iscshemic hypoxia in the brain (Table 1). In addition, the area of infarction generated in this model is very limited, thus limiting its effectiveness in stroke-related studies (Kumar *et al*., 2016).

Table 1. The use of global ischemic animal models in ischemic stroke research

Method	Species	Procedure	Advantages	Disadvantages	Reference
2-VO model	Rats	Bilateral communist carotid artery occlusion that causes memory impairment	Easy procedure as well as only one day of operation	Poor reproducibility, Induction of hypotension	(Chen *et al*., 2019; Pontarelli *et al*., 2012)
4-VO	Rats	On the first day,	Easy to set up,	Limited surgical	(Lu *et al*.,

Method	Species	Procedure	Advantages	Disadvantages	Reference
model		permanent occlusion of the vertebral artery was performed using electrocoagulation, which was then followed by a temporary blockage of the communist carotid artery the next day.	high consistency of results, and low incidence of seizures.	expertise, high mortality	2016; Li & Zhang, 2021).
Cardiac arrest	Rats	Electrocution of the heart with electrical stimulation	It bears a strong resemblance to the clinical state of heart attack and resuscitation in humans.	Extracerebral complications, complex procedures, and low survival rate.	(Dave *et al*., 2013)
Chemical and gas hypoxia	Zebrafish	Blockage of brain blood vessels with chemicals and hypoxia gases	It is easy to do and effective to use in the initial evaluation of drugs for stroke therapy.	Limited to simulation of hypoxia mechanisms in the brain.	(Marino *et al*., 2020)

3.1.2 Focal ischemic model

The focal ischemic model is the most used because it has a higher relevance to ischemic conditions in humans. Because middle cerebral artery (MCA) disorders typically cause ischemic stroke in humans, MCA blockage is often used as a primary model in pre-clinical stroke research (Howells *et al*., 2014). Here are some methods in the focal ischemic model.

a) Intraluminal MCAO (Middle Cerebral Artery Occlusion)

This model is widely recognized as the main reference in research examining the mechanisms of ischemic stroke occurrence (Herson & Traystman, 2014). Research by Howells et al (2014), stated that this model was used in more than 40% in 2,582 neurological protection trials. Technically, the MCAO model can avoid damage to the skull structure. This procedure is performed by inducing temporary occlusion in the communist carotid artery through the insertion of sewing thread into the inner carotid artery, which is then directed forward until it stops blood flow to the artery (**Table 2**) (Fluri *et al*., 2015).

This model is ideal for researching the restoration of neurological function in response to therapies that stimulate nerve regeneration and improve neuronal plasticity. However, the downside of this model is that severe injuries can lead to animal death, especially after permanent or long-lasting temporary MCA occlusion (**Table 2**), which is usually caused by the

formation of brain edema associated with stroke, dehydration, malnutrition, and/or infectious conditions (Hermann *et al*., 2019).

b) Endothelin-1 induced stroke (ET-1)

Focal induction of endothelin-1 into the blood vessels of the brain results in ischemic reperfusion injuries like human strokes and can minimize mortality in animals. As for the disadvantages it can provoke lesions due to vascular response to Endothelin-1 (ET-1) (Soeandy *et al*., 2019). Based on the research of Ansari *et al.* (2013), it is still unknown exactly how large the penumbra network this model produces. However, several studies have applied this model and shown that certain therapeutic approaches can reduce the volume of infarctions and potentially repair damaged tissues.

One of the advantages of this model is that surgery is relatively fast and easy to target MCA, Low invasion rate and low mortality rate. The disadvantage is that the duration of ischemia cannot be controlled, variations in the way blood vessels react to ET-1 may be the source of large diversity in lesion volume (**Table 2**). Induction of astrocytosis and axon growth that can complicate the interpretation of results (McCabe *et al*., 2018).

c) Electrocoagulation model of MCAO

This model is used to coagulate certain parts of the MCA to permanently close blood vessels (Ma *et al*., 2012). After a clot occurs in the blood vessel, the researcher will cut off the part that has been frozen for a thorough occlusion can be guaranteed. One of the advantages of this model is the flexibility in determining the location of occlusion in the MCA, both in the distal and proximal parts, so that it can produce strokes that are limited to the cortical area only or include the cortical and subcortical areas (Tarr *et al*., 2013).

This model offers a high rate of reproducibility and generally shows a lower variation in lesion size compared to the MCAO model using filament. Nonetheless, the application of this model demands higher surgical skills, especially during craniectomy, when MCA is exposed without causing bleeding or significant damage to the cortex underneath. In addition, due to the location of the cranectomy procedure, there is a risk of damage to the temporal muscles, especially when occlusion is performed in the proximal part (**Table 2**) (Tarr *et al*., 2013).

d) Cerebral photothrombosis

In this model, proximal occlusion of MCA was induced using rose bengal in mice, which then triggered the formation of lesions in the cortical and subcortical regions (Qian *et al*., 2016). As for the advantages of this model is that the surgery can be completed quickly, it does

not require complex surgical techniques, so additional trauma due to craniotomy can be avoided. This results in a moderate level of neurological deficit, accompanied by a low mortality rate and a high rate of reproducibility. The disadvantages of this model are the lack of penumbra and poor response to thrombolytic therapy (**Table 2**).

The cerebral photothrombosis model is not suitable for research related to neuroprotective drugs. This is due to the microvascular clot that forms, which is very rich in platelets, so it can only be partially overcome by recombinant tissue plasminogen activators. In addition, the resulting brain infarction often indicates the presence of bleeding around the core area of the lesion. Consequently, this model is also not the best for assessing how well thrombolytic medications work (Qian *et al*., 2016).

e) Cerebral thromboembolism

This model allows for research into thrombolytic agents, including their ability to dissolve blood clots, reopen blocked blood vessels and most importantly allow testing of potential new neuroprotective agents in conjunction with thrombolytic therapy (Chen *et al*., 2019). The high rate of partial and complete reperfusion experienced by 80% of animals after three hours of blockage is a weakness of the model (Durand *et al*., 2012). However, late administration does not have a damaging effect (Orset *et al*., 2016).

The cerebral thromboembolism model is considered the most clinically relevant because it resembles a stroke in humans. However, the procedure is complex, requires high microsurgical skills, and results in brain infarctions with greater variation than intraluminal MCA occlusion models (**Table 2**).

Table 2. The use of ischemic focal animal models in ischemic stroke research

Method	Species	Procedure	Advantages	Disadvantages	Reference
Intraluminal MCAO	Mice	Inserting the sewing thread directly into the internal carotid artery, the thread is forced to stop the MCA blood supply.	The most widely used models, very similar to human ischemic stroke	Some animal deaths mainly after longer/ permanent MCA occlusion, a large degree of injury	(Cai *et al.*, 2016)
Endothelin-1 induced stroke	Rats, monkey	Focal application of endothelin-1 to brain blood vessels	Produces ischemic reperfusion injuries like human strokes, resulting in reproducible tissue injury, Improved survival	The duration of ischemia cannot be controlled, and astrocyte activation and axon branching can complicate data	(Soeandy *et al*., 2019; Dai *et al*., 2017; Zhang *et al*., 2016)

Method	Species	Procedure	Advantages	Disadvantages	Reference
				interpretation	
Electrocoagulation model of MCAO	Mice	Blood flow is permanently stopped by freezing a specific part of the MCA using electrocoagulating forceps	Stable, able to show a larger volume of infarction	Permanent MCAO induction does not allow reperfusion and requires more complex surgical skills.	(Ma *et al*., 2012)
Cerebral photothrombosis	Rats	The proximal cerebral artery was blocked using a laser after intravenous administration of rose bengal to mices	The operation can be completed quickly, no professional surgical techniques are required, high reproducibility	Lack of penumbra; Cerebral infarction shows bleeding around the nucleus of the lesion, limited use for acute stroke	(Qian *et al*., 2016)
Cerebral thromboembolism	Rats	Autologous thrombus is directly induced inside MCA with Microinjections produce infarction volume	suitable for thrombolysis studies, resembling cerebral in humans	Minimal craniotomy was performed to expose MCA, resulting in a brain infarction with greater variation	(Chen *et al*., 2019; Dotson *et al*., 2016)

3.2. Parameters of ischemic stroke

3.2.1 Haematology

According to Donkel *et al.* (2019), some of the biomarkers used in ischemic stroke research include coagulation/thrombosis biomarkers consisting of fibrinogen, D-Dimer, β-Thromboglobulin, Platelet factor-4, Mean platelet volume (MPV), platelet count, Antithrombin III (**Table 3**). However, there are no definite recommendations regarding clinical outcomes after acute ischemic stroke. However, several biomarkers show promising potential and still require further research.

Table 3. Ischemic stroke hematological biomarkers

Biomarker	Description	Reference
Fibrinogen (Fg)	Identifying the etiological subtypes in acute ischemic stroke is essential, especially in estimating the potential for cardioembolism	(Liu *et al*., 2015)
D-Dimer	Fibrin braiding breakdown products after factor XII stabilization; Indications of thrombus formation.	(Whiteley *et al*., 2008)
β-Thromboglobulin	Increased plasma b-TG levels increase platelet aggregation and further accelerate thrombosis.	(Cui *et al*., 2012)
Platelet count, Platelet	Platelet count is a reliable predictor of mortality	(Yang *et al*.,

Biomarker	Description	Reference
factor-4, Mean Platelet Volume (MPV)	rates, poor functional outcomes, and the risk of chronic stroke recurrence.	2019)
Antithrombin III	Antithrombin III levels were shown to be significantly decreased throughout the measurement time after the occurrence of stroke	(Muchti *et al*., 2019)

A few studies indicate that high levels of fibrinogen, D-Dimer, and b-TG are correlated with an increased risk of stroke compared to lower levels. In ischemic stroke patients, fibrinogen levels may slightly increase or remain normal, and the differences between stroke subtypes tend to be insignificant. However, as an acute phase protein, increased fibrinogen levels are also associated with the magnitude of the volume of brain infarctions. D-Dimer itself is the end product of the process of fibrin degradation due to hydrolysis by plasmin after the occurrence of cross-binding between fibrin monomers and thrombin activator XIII, and serves as a specific marker of fibrinolysis activity (**Table 3**) (Liu *et al*., 2015). As for β-Thromboglobulin, increased plasma b-TG levels increase platelet aggregation and further accelerate thrombosis (Cui *et al*., 2012).

Some studies have shown that platelet counts and antithrombin III are significantly lower in patients with ischemic stroke (**Table 3**). In focal cerebral ischemia, disturbances in the hemostasis system may occur, involves observable platelet activation, especially during the acute phase when fibrinolysis decreases and thrombin activity increases. One of the important steps in the coagulation mechanism is the transformation of prothrombin into active thrombin (Muchti *et al*., 2019).

3.2.2 Biochemistry

According to Alkireidmi *et al.* (2018), biochemical parameters such as enolase serum, CK-BB, LDH, and lipid profiles can be effective biomarkers for ischemic stroke because the tests are simple, inexpensive, and fast (**Table 4**). Enolase serum is considered the most sensitive and specific as a diagnostic marker of ischemic stroke.

Table 4. Biochemical biomarkers of ischemic stroke

Biomarker	Description	Reference
Enolase serum	Acute ischemic stroke can be effectively treated with the use of biological markers that indicate acute nerve cell damage. Serum enolase levels are expected to increase as infarction progresses	(Shash *et al*., 2021)
CK-BB activity	The activity of Creatine Kinase (CK) in serum in patients with acute ischemic stroke may be related to the severity of the condition. Patients with neurological conditions show an increase in CK-BB	(Alam *et al*., 2014)
Lipid profile, total	Lipids have a complex relationship with ischemic stroke	(Yaghi & Elkind,

Biomarker	Description	Reference
cholesterol; LDL; HDL; triglycerides	disease. Higher total cholesterol is associated with an increased risk of ischemic stroke. Atherosclerotic is strongly related to TG and LDL	2015)

Various enzymes are found in high amounts in the brain, and conditions such as stroke can trigger an increase in the work of these enzymes in the cerebrospinal fluid. The increase in the level of enzymes in serum generally depends on the integrity of the brain's blood barrier. If the damage is severe enough to interfere with the barrier, then the enzyme activity in the serum is likely to increase (Alam *et al*., 2014).

The serum enolase concentration and average CK-BB activity in stroke patients were significantly higher. Serum enolase levels are expected to increase as infarction progresses. Thus, in the case of acute ischemic stroke, serum enolase acts as a strong biochemical indicator to predict functional outcomes. The study also revealed that although the size of the infarction was difficult to measure precisely, serum enolase levels in the early stages had a strong correlation with stroke severity (Shash *et al*., 2021). In addition, in the stroke patient group, there was a correlation between the severity of brain damage, as shown by clinical images and CT scans, and serum BB-CK values (Alam *et al*., 2014).

Lipids play a complex role in cerebrovascular diseases. There is a direct relationship between cholesterol levels, especially total cholesterol (TC) and LDL, with ischemic stroke and atherosclerosis. On the other hand, there is a link between low cholesterol and an increased risk of intracerebral hemorrhage (ICH) and a higher likelihood of developing small blood vessel disease (Yaghi & Elkind, 2015).

3.2.3 Cytology

Cytology is a field related to the science that studies the morphology of cells individually or cells derived from tissue fragments observed microscopically. The parts of the cell have their own functions. Mitochondria play a fundamental role in regulating cell survival, both towards cellular survival and death. These organelles serve as the main centers of energy production and are present in large quantities in brain cells (Fidaleo *et al*., 2017). Therefore, it is related to the incidence of ischemic stroke. In addition to mitochondria, several parts of the cell that affect ischemic stroke are described below.

a) Mitochondrial

Mitochondrial dysfunction is seen as a leading indicator of neuronal death caused by ischemia (Liu *et al*., 2018). Ischemic stroke causes oxidative stress due to the buildup of calcium

ions and reactive oxygen species (ROS) in cells, which then disrupts mitochondrial function and activates apoptosis factors, leading to apoptosis (Andrabi *et al*., 2020). The following is the mechanism of ischemic stroke due to mitochondrial dysfunction (Liu *et al*., 2018):

1) Mitochondrial response: increased ROS production, excess calcium accumulation in the mitochondria, and disruption of mitochondrial quality control mechanisms.
2) Excitotocity: excessive excretion of glutamate accompanied by inhibition of the mechanism of exitatory amino acid reuptake contributes to the activation of ionotropic receptors such as NMDAR, AMPAR, and KAR.
3) Asidotoksitas: neuronal death due to ischemia can be triggered by an extracellular pH drop that activates acid-sensitive ion channels 1a.
4) Cerebral ischemia triggers misfolding and aggregation of proteins that can contribute to cellular dysfunction.
5) Inflammatory: the activation of microglia is accompanied by the secretion of proinflammatory cytokines and chemokines that trigger an immune response, which further initiates various mechanisms of cell death.

b) Endoplasmic reticulum

As a major center of protein synthesis and intracellular calcium balance regulator, the endoplasmic reticulum undergoes a stress response because of various neurological dysfunctions. Ca^{2+} imbalances, in the form of excess in the cytosol and deficiency in the RE lumen, can trigger cellular stress and protein misfolding. Neurons, glia cells, and endothelials play a role in the pathophysiology of ischemic strokes. Several proteins during cerebral ischemia involve a series of specific inflammatory and apoptosis signaling pathways (Han *et al*., 2021).

c) Lysosomes

As the main organelle in charge of cleaning proteins and damaged organelles, lysosomes play a crucial role in maintaining intracellular homeostasis in neurons. TMEM175 is known as the new K+ channel which plays a role in regulating the lysosomal membrane potential and maintaining pH stability within neurons. TMEM175 deficiency caused by cerebral ischemic injury causes lysosomal pH stability to be disrupted, thereby inhibiting lysosomal hydrolytic function. As a result, the degradation of damaged mitochondria that depend on lysosomes and thus worsens brain damage (Zhang *et al*., 2020).

d) Ribosomal

Based on research, ribosomes may play a role in post-stroke peripheral immunosuppression, which could be an important mechanism in determining ischemic stroke outcomes. Ribosomal protein S14, S15A, S24, L27, L31, L34, L35A, L24D1 (Ribosomal L24 domain containing 1), FAU (FAU ubiquitin like and ribosomal protein S30 fusion), and EEF1B2 (Eukaryotic translation elongation factor 1 beta 2) potential to be a new prognostic biomarker as well as a therapeutic target for stroke (Xie *et al*., 2020).

3.2.4 Histological

Histological biomarkers in stroke play an important role in distinguishing between strokes caused by cardioembolic and noncardioembolic. The histopathological analysis of the embolistic clot aims to identify specific patterns that can help distinguish the cause of ischemic stroke. First, an evaluation of the basic identifiable morphology of fibrin/platelets and red and white blood cells. Second, immunohistochemistry tests, including analysis of differentiated coreceptor groups such as CD3, CD20, and CD68/KiM1P (Sporns *et al*., 2017).

3.2.5 Molecular

The molecular biomarkers of stroke consist of biomarkers of proteins, amino acids and enzymes, inflammation, neurotrophic and growth factors. For example, in proteins are S100β, GFAP, AQP4, FABPs. In amino acids and enzymes such as serine, glutamate, Glutamine synthetase, Serine racemase. Inflammatory mediators such as Interleukin-6 (Il-6), Interleukin-4 (Il-4), MMP-9, Tumor Necrosis Factor α (TNF-α), as well as neurotrophic and growth factors such as Brain-Derived Neurotrophic Factor (BDNF), Glial Cell Line-Derived Neurotrophic Factor (GDNF), and Nerve Growth Factor (NGF), play an important role in the regulation of cellular responses to nerve injury (Steliga *et al*., 2020).

4. Conclusion

The test animal model in the ischemic stroke experiment consisted of a focal ischemic model and a global ischemic model. The focal ischemic model is more relevant to ischemic stroke in humans than the global ischemic model. The various types of animal models that can be used have their own advantages and disadvantages, so it is important to choose the right model to produce reliable and valid research in ischemic stroke testing. In addition, several parameters such as hematology, biochemistry, cytology, histology, molecular with their

biomarkers can be selected to help in identifying the incidence of ischemic stroke in test animals.

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