

**Original article:**

**Analysis of TGF- $\beta$ 1 and p53 Expression in The Blood of Stroke Patient**

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**Abstract**

**Introduction:** Stroke is a dysfunction of brain tissue due to deficiency of blood circulation to the brain. This causes disruption of blood supply and oxygen in the brain. Stroke not only needs to be handled in post-event, but also needs to be understood the cause of the occurrence by molecular, so that the drug will be right on target. Inflammation is associated as a secondary injury mechanism in the case of stroke. Dendritic cells which secreted TGF- $\beta$ , it functions to regulate proliferation, differentiation and activate other cytokines in cell growth. It is strongly suspected that Transforming Growth Factor- $\beta$ 1 (TGF- $\beta$ 1) is a determinant of cytokines in nerve tissue recovery. Neural cell malfunction or nerve cell death is thought to be the role of p53 protein. **Methods:** The design of this research is observation. This research measures TGF and p53 expression in the stages of mRNA (RT-PCR) and protein (ELISA) in the blood of stroke patients. **Results:** The expression of TGF-  $\beta$ 1 and p53 mRNA in the blood of stroke patients is higher (50 and 45 times; Unpaired p <0.01) than normal. TGF-  $\beta$  and p53 protein levels in the blood of stroke patients are higher than normal (20,607 vs 1,895 pg/mLx10; 44,418 vs 11.63 pg/mL; Unpaired 0 <0.01). The correlation of both TGF-  $\beta$ 1 and p53 shows a strong positive. **Discussion:** The expression of increased TGF-  $\beta$ 1 and p53 In the blood of stroke patients. **Keyword:** Stroke, TGF-  $\beta$ 1, inflammation, p53, blood.

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**Introduction:**

Strokes occur due to dysfunction of brain tissue which results in reduced blood and oxygen supply to the brain. Symptoms of a stroke can take place very quickly, can last for 24 hours and even sudden death can occur. In developing countries, there are around 5.8 million people who die suddenly from stroke. In the world, stroke is the third leading cause of death after ischemic heart disease (IHD) and cancer. Ischemic symptoms dominate 87% of stroke case. Ischemic stroke is a multifactorial. Strokes occur due to dysfunction of brain tissue which results in reduced blood and oxygen supply to the brain. Symptoms of a stroke can take place very quickly, can last for 24 hours and even sudden death can occur. In developing countries, there are around 5.8 million people who die suddenly from stroke.<sup>1,2</sup> In the world, stroke is the third leading

cause of death after ischemic heart disease (IHD) and cancer. Ischemic symptoms dominate 87% of rial disease that involves the interaction of various genetic and environmental factors. Inflammation plays an important role in the process of the pathogenesis of ischemic stroke,<sup>3</sup> until now still needs further study of literature and research. Stroke is vulnerable in the old age group, due to frequent occurrence of blood circulation disorders due to changes in degenerative cells and atherosclerosis.<sup>4</sup>

Ischemic stroke can also occur at productive age, this is due to increased activity and stress at work. Besides the cause of stroke is preceded by previous diseases such as hypertension, diabetes mellitus, and heart. The disease is a major risk factor for stroke. In patients with hypertension, increased blood pressure can weaken the walls of blood

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vessels and damage the inside of blood vessels. This can lead to the formation of atherosclerotic plaque. These events facilitate the occurrence of brain blockage or bleeding.<sup>5</sup> Parathyroid hormone (PTH) also plays a role in plaque stability in blood vessels.<sup>6</sup> Increased lipid components in the blood in the case of stroke are ceramide and phospholipid lipids and followed by decreased apolipoprotein.<sup>7</sup> In the results of previous studies there were no differences in lipid profiles in new or recurrent ischemic strokes.<sup>8</sup> Other information states that increasing the S-100 protein component can reduce nerve sensitivity of cells in cases of stroke.<sup>9</sup>

Stroke not only needs to be treated post-event, but also needs to be understood the cause of the occurrence by using molecular parameters, so that the drug will be given right on target. There have been many previous research efforts in stroke patients that have been carried out, including improving the mechanism of regulation of the system in the brain is regulated through an increase in the hypothalamus-pituitary-adrenal (HPA), then subsequently will increase the secretion of adrenocorticotrophic hormone (ACTH). This increase in HPA is also influenced by cytokine proteins in the form of interleukin-6 (IL-6) and TNF- $\alpha$ .<sup>10</sup> MicroRNAs (miRNAs) which are small molecules of non-coding RNA. MicroRNAs are involved in many physiological processes whose role as posttranscription inhibitors of gene expression in forming enzyme proteins that form atherosclerosis in cases of stroke.<sup>11</sup> These results are not significant, so further research is needed.

In the case of stroke there is also an increase in T cells, Interleukin-10 (IL-10), Interleukin-6 (IL-6) and change growth factor-beta (TGF- $\beta$ ) in 1-3 weeks post-stroke,<sup>9</sup> it is accompanied by the emergence of inflammatory cells that play an important role in determining the pathophysiology of stroke.<sup>12</sup> This T cell regulation is important as a major protective immunomodulator in early post stroke (neuroprotectat). This inflammatory mechanism is likely to be the target of drugs and various biomarkers.<sup>13</sup> This information still needs further research on the types of inflammatory cells involved in and their regulatory mechanisms.

Inflammation is associated as a secondary injury mechanism in the case of stroke. Various experimental models, including thromboembolic stroke, focal and global ischemia, have been used to evaluate the occurrence of inflammation in stroke. Endothelium vascularization promotes

inflammation through upregulation of adhesion molecules by ICAM, E-selectin, and P-selectin proteins bound to leukocytes either circulating or migrating to the central nervous system (CNS). The production of inflammatory proteins that are toxic in the CNS, can cause death cell. Macrophage and microglial responses to inflammatory responses are useful for detecting the occurrence of necrosis or cell death in neurons so they can be recovered. However, further research on the role of blood vessels, leukocytes, brain blood barrier, macrophages and microglia are still needed.<sup>14</sup>

Transforming Growth Factor Beta-1 (TGF- $\beta$ 1) is a pleiotropic cytokine that has strong anti-inflammatory properties, and is also considered a cause of ischemic stroke inflammation by involving various components of the pathophysiological process including lipid metabolism, hypertension and osteosclerosis. The TGF- $\beta$ 1 gene is located on chromosome 19 (q13.1–13.3), including 7 exons and 6 introns. TGF- $\beta$ 1 protein expression is largely under the genetic control of the TGF- $\beta$ 1 gene. General SNP in this gene can modify TGF- $\beta$ 1 protein expression and TGF- $\beta$ 1 genetic polymorphism is associated with an increase in serum TGF- $\beta$ 1 levels.<sup>15</sup> Ischemic stroke also involves cell death from several cellular components including neurons and glia cells associated with TGF- $\beta$ 1 regulation. It is suspected that p53 tumor suppressor also plays an important role in regulating cell death in stroke cases. P53 protein is reported to be regulated after ischemic injury and leads to apoptosis in the ischemic area. P53 protein is an important mediator of programmed cell death in the ischemic region. Inhibition of p53 gene activity by pharmacological inhibitors and consequently eliminate p53 function in all cells in the ischemic area. Past studies have not yet answered the function of p53 in regulating neuronal cell death in ischemic injury.<sup>16,17</sup>

The inflammatory process that underlies stroke involves the role of TGF- $\beta$ 1 and p53, but the correlation of the two proteins is unknown. This research aims to analyze the expression of TGF- $\beta$ 1 and p53 in an effort to develop molecular parameters of the process of stroke, as a diagnostic basis for treatment targets.

#### **Methods and Materials:**

The study design was descriptive observational. Blood was obtained from 10 stroke patients (who were not infected) through clinical procedures in the hospital and as control subjects came from 10 healthy people. The research parameters

detected TGF- $\beta$ 1 and p53 expression. Sampling of stroke patients takes place from January to March 2019 at the National Brain Central Hospital. This research has been through an ethical review through the Ethics Committee, Faculty of Medicine, Syarif Hidayatullah State Islamic University approved of this study (ethical permit No. 02/FK-UINSH/ETIK/2018). The materials used in our study are: for RT-PCR techniques: TGF- $\beta$ 1 and P53 primers, WHY SYBR QUICK one-step qRT-PCR universal Kapa Biosystems (KK4650), mini isolation RNA Kit (Geneaid Biotech. Ltd), Water-Biotechnology Gradesterilized-Free protease and pyrogen free (BUF-1180),  $\beta$ -mercaptoethanol; for ELISA and techniques: Human TGF- $\beta$ 1 and ELISA Kit Cusabio (CSB-E12112h), Human TGF- $\beta$ 1 and p53 ELISA Kit Cusabio, Phosphate Saline Buffer 7.4.

Isolation of RNA from blood samples isolated using a mini RNA Kit (Geneaid Biotech. Ltd) and purity measured with a ratio of A260 / A280 > 1.7. This research synthesizes cDNA amplification by PCR; with KAPA® SYBR QUICK one-step universal qRT-PCR (KAPA Biosystems). The human TGF- $\beta$ 1 and P53 sequences were tracked through the NCBI-Gene bank. The primers used for TGF- $\beta$ 1 are: advanced 5'-CTCGTGGATCCACTTCCAG-3', reversing 5'-CTCGTGGATCCACTTCCAG-3', 120 bp products Primer for p53: advanced 5'-CACTGCCCAACAACACCAGCCCCT-3', reverse 5'-GTCCGCTCCAGCT 3', 120 bp products Primer for p53: advanced 5'-CACTGCCCAACAACACCAGCCCCT-3', reverse 5'-GTCTGAGCTCTCT. Primer used for 18S: advanced 5'-AGAAACGGCTACCACATCCA-3, reversing 5'-CCCTCCAATGGATCCTCGTT-3, product 258 bp. For the purpose of this RT-PCR analysis, RNA samples were diluted to 200 ng /  $\mu$ L, with a temperature melting curve (Tm) for TGF- $\beta$ 1 of 84oC, p53 and 18S of 80o C. The mRNA level was measured according to the Livack method.

ELISA technique for detecting TGF- $\beta$ 1 and p53 protein levels. TGF- $\beta$ 1 and p53 protein levels were measured using the ELISA Kit Cusabio. The steps are as follows: the method starts with making a standard protein, with a concentration of 0; 0.0625; 0.125; 0.25; 0.5 (ng/mL). Microplates are prepared, which have been coated with primary antibodies.

About 100  $\mu$ L of each serum, sample and standard

were transferred to microplates and incubated for 2 hours at 37°C. After incubation, the supernatant is removed and the well is rinsed 3 times with Wash Buffer. About 100  $\mu$ L HRP-avidin was added and incubated for 1 hour at 37°C. The supernatant is removed and the well is rinsed 5 times with Wash Buffer. About 90  $\mu$ L TMB-substrate was transferred to the well and incubated in a dark room for 15-30 minutes at 37°C. Next, 50  $\mu$ L stop solution was added, the color results were read using an ELISA reader at a wavelength of 450 nm.

### Results:

Study on mRNA expression using RT PCR we carried out the calculation of each sample in Duplo. The Livak method of calculation, we found the expression on TGF- $\beta$ 1 mRNA of stroke is  $50 \pm 8.2$  times to normal; (Unpaired t-test;  $P = 0.000$ ) (figure 1A). We found the expression on P53 mRNA of stroke is  $42 \pm 3.2$  times to normal (Unpaired t-test;  $P = 0.000$ ) (figure 1B) in accordance to the Livak method of calculation. The correlation between mRNA TGF- $\beta$ 1 and p53 shows strong positive and significant correlation ( $R = 0.649$ ;  $P = 0,000$ ) (Figure 1.C).

Assessment of protein level using ELISA showed that TGF- $\beta$ 1 protein in the normal was in  $18.95 \pm 1.1$  pg/mL and in stroke was in  $206.07 \pm 7.1$  pg/mL (Unpaired t-test;  $P = 0.004$ ) (figure 1A). p53 protein of normal were in  $116.3 \pm 14.8$  pg/mL and stroke were in ranged of  $444.18 \pm 4.8$  pg/mL (Unpaired t-test;  $P = 0.004$ ) (figure 1B). We found elevated of p53 protein was in accordance with TGF- $\beta$ 1 protein (figure 1C).

### Discussion:

Protein growth factor- $\beta$ 1 transformation (TGF- $\beta$ 1) is a multifunctional cytokine, whose function in nerve cells is increasingly recognized. TGF- $\beta$ 1 signals, including serine kinase-type transmembrane receptors, are present in the central nervous system. There are 3 subtypes of TGF- $\beta$  mammals that have different distribution and function in the brain and nerves. Their involvement in the development and plasticity of the nervous system in peripheral organs shows as a neuroprotective function. Indeed, TGF- $\beta$ -induced expression appears following various types of brain tissue injury. The nerve protection function by TGF- $\beta$  most often occurs after brain ischemia.<sup>16</sup> The TGF- $\beta$ 1 expression has been reported in microglia and astrocytes.

Excessive expression of TGF- $\beta$ 1 in the adenoviral brain that is protected by adenoviral vectors in ischemic stroke. This TGF- $\beta$ 1 can reduced

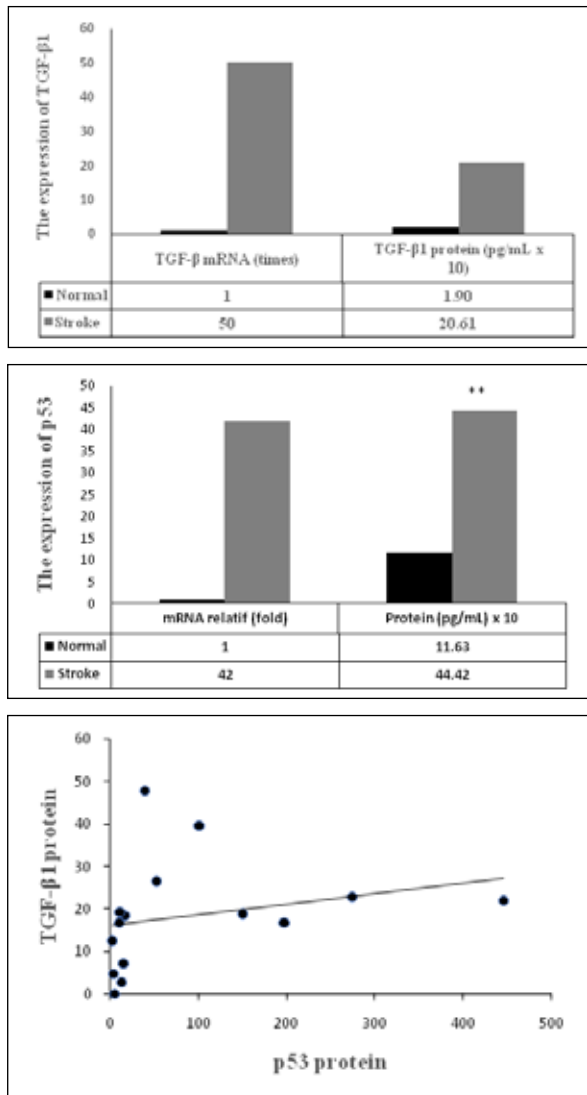


Figure 1. (A) The expression of TGF-β1 mRNA and protein in blood of stroke (B) The expression p53 mRNA and protein in blood of stroke (Unpaired t-test  $p < 0.01$ ). (C) The correlation between mRNA of TGF-β1 and p53 in keloid (Pearson,  $R = 0.649$ ;  $P = 0.000$ ).

the accompanying inflammatory response. A recent study showed that neurons that can be protected from ischemia by secreted microglia-TGF-β. In this case TGF-β excels in the recovery process of several CNS diseases. It can be recommended that TGF-β plays a role in the recovery process of ischemic stroke. Basically an important inflammatory response accompanies necrotic brain injury. Acutely, this inflammatory response contributes to the level of ischemic pathology. This will show the role of specific cell types such as leukocytes, endothelium, glia, microglia, extracellular matrix, and neurons. Likewise intracellular inflammatory signaling pathways such as Nuclear factor κB (NF-κB) and

Mitogen-activated protein kinases (MAPK), and mediators produced by inflammatory cells such as cytokines, chemokines, reactive oxygen species and arachidonic acid metabolites and therapeutic potentials that cause stroke and hypoxic-ischemic injury. The target of the drug in this case is an attempt to stimulate the emergence of TGF-β1 so that inflammation is not sustainable.<sup>17</sup> The role of TGF-β in this case is to stimulate the T cell differentiation process, thereby reducing the inflammatory process for stroke recovery.<sup>18</sup>

The tumor-suppressor protein p53 in nerve cells play a role in cellular functions including cell proliferation, stimulating cell death, genome stability, and regulation of inflammation. In nerve inflammation through the picture of the central nervous system (CNS), immune response begins with microglia in a population of CNS myeloid cells. Microglia maintain CNS homeostasis through the arrest of pathogens by expressing p53, followed by phagocytosis debris, and initiation of the cascade in tissue repair. The presence of p53 regulates the pro-inflammatory response which causes tissue injury and dysfunction in acute and chronic inflammatory states. Therefore, this molecular signal regulation is needed for optimal CNS health.<sup>19</sup>

During cerebral ischemia, p53 plays a role in stimulating macrophages to carry out their functions in the anti-inflammatory M2. M1 macrophages have inflammatory properties producing inflammatory mediators, such as IL-1β, TNF-α, and chemokines (eg MCP-1, MIP-1α), while M2 macrophages have anti-inflammatory properties by producing anti-inflammatory cytokines, such as IL-10 and transforming growth factor beta-1 (TGF-β1).<sup>9</sup>

TGF-β1 influences cell survival, and as an anti-apoptotic effect on neurons through p53 regulation. Nerve regeneration can also involve TGF-β1 during post-inflammatory tissue recovery. The role of TGF-β1 varies depending on the type of brain lesion. The role of nerve inflammation occurs in various brain pathologies, including ischemic and traumatic brain injury. Neurodegenerative disorders can also include aspects of inflammation. In turn, pathogens often develop ways to protect against hosts that have inflamed defense mechanisms. Therefore. This suggests TGF-β1 may not be very effective in infection.<sup>17</sup> Inflammation is a double-edged sword that is responsible for the pathogenesis of ischemic stroke and other forms of ischemic

brain injury and also contributes favorably to post-stroke brain recovery. TGF- $\beta$ 1 manages its work through this post-stroke start process. The modification of TGF- $\beta$ 1 in which three isoforms can bind to the same receptor, through regulation of focal cerebral ischemia and TGF- $\beta$ 1 mRNA increases within 1-6 hours and remains up to 15 days after stroke. In this case TGF- $\beta$ 1 plays an important role as a neuroprotective and anti-inflammatory in stroke and is recommended as an effective therapeutic agent for stroke. When TGF- $\beta$ 1 expression is increased it can reduce ischemic injury and reduce the accompanying inflammation. Inhibition of TGF- $\beta$  exacerbates ischemic brain damage. However, other data have reported an insignificant role when TGF- $\beta$ 1 is given after stroke recovery. This may be related to mismatch of area requirements, because TGF- $\beta$ 1 is shown to have a neuroprotective role when in the penumbra area, but there are no beneficial effects when in the core area.<sup>9</sup>

The role of p53 in the mechanism of apoptosis is prominent in cerebral ischemia and traumatic brain injury around the area of primary damage. This area is clinically because the opportunity to sustain cell damage does not continue during the treatment period. The anti-apoptotic and anti-excitotoxic effects of TGF- $\beta$ 1 can be relevant when aiming to develop a protective agent against nerves in the post-inflammatory recovery process. Furthermore, in occlusion of brain vessels during stroke followed by proliferation of micro vessels, TGF- $\beta$ 1 stimulates angiogenesis. This process is also specifically marked in the border zone of the infarction, ischemic penumbra, where there is a decrease in blood flow after focal ischemia. This increase in vascularization is another process, in which TGF- $\beta$ 1 can stimulate increased blood flow to the brain after an injury occurs. Increased blood flow in this vulnerable area can reduce the occurrence of ischemic attacks again.<sup>16</sup>

The thing that is different, in the case of ischemic

stroke there is also an increase in ROS production and activates the Fas death receptor which stimulates the activation of caspase-8 and p53 pro-apoptosis. Increased ROS results can stimulate DNA damage and increase the mechanism of p53 phosphorylation that activates cell death pathways.<sup>18</sup> The binding of p53 protein in the process of apoptosis by early nerve progenitor cells, shows that p53 expression selectively induces apoptosis widely by increasing p53 levels and increasing transcription of pro-apoptotic target genes, such as Bax and Noxa. In the apoptotic target area that has been accomplished or fulfilled, p53 is maintained at a low level with ubiquitination, proteasome degradation and stabilized cellular recovery mechanisms. Protein p53 protein is also involved in cellular responses to ischemia. The stability of p53 expression depends on the severity and duration of hypoxia/ischemia. This will have a very different effect on the activity, level, and function of p53 apoptosis.<sup>20</sup>

#### **Limitations and Problems:**

The number of samples is small, the blood sample of stroke patients is difficult to obtain, so to get it waiting for routine laboratory examinations in the emergency department.

#### **Ethical Approval:**

The Committee of Ethic, Faculty of Medicine, State Islamic University Syarif Hidayatullah approved the study (ethical clearance No. 02/FK-UINSH/ETIK/2018).

**Conflict of interest:** None declared

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#### **Author's Contributions:**

Data gathering and idea owner of this study:RAF

Study design: RAF

Data gathering: HB

Data analysis and consultation: EW Writing and submitting manuscript: EW

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