



## Plasma Vascular Endothelial Growth Factor (VEGF) In Ischemic Stroke – A Comparative Study

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### ABSTRACT

**Background:** *Studies done elsewhere in stroke have shown that there is elevated VEGF in patients with ischemic stroke and it can be used as a biomarker in ischemic stroke for early diagnosis and treatment. But human studies are limited in our set up. Hence the present study is carried out to compare the level of VEGF in ischemic stroke patients and asymptomatic individuals from general population.*

**Aim:** *To compare the level of Plasma VEGF level in patients with ischemic stroke with equal number of age and sex matched normal individuals without stroke.*

**Materials and Methods:** *Hospital based cross sectional study. Newly diagnosed ischemic stroke patients, diagnosed by CT scan and clinical signs and symptoms in the age group 40 to 65 years admitted in The Department of Neurology were included as cases , Equal number of age and sex matched individuals without stroke from general population were also included for comparison. The sample size was calculated to be 24 patients and 24 controls. After taking informed consent, 5ml fasting blood samples drawn into plain and EDTA –Na F containing bottles from antecubital vein. Plasma VEGF was determined by ELISA method. Statistical analysis was performed using SPSS for windows version 20. The mean and standard deviation for quantitative variables and percentage for qualitative variables were calculated for 24 ischemic stroke patients and 24 age and sex matched asymptomatic individuals from general population. Differences in means of quantitative variables between the two groups were compared by student t test. Chi square test was used to compare differences in the percentage of qualitative variables between the groups .A P value of less than 0.05 is considered significant.*

**Results:** *Plasma VEGF (Vascular Endothelial Growth Factor) is increased in the stroke group with mean value  $245.52 \pm 91.57$  pg / ml compared to the mean VEGF in non-stroke individuals, which is  $103.32 \pm 51.65$  pg / ml. Student t test was done to analyze the difference in mean VEGF levels between these groups and the difference was found to be statistically significant (p value < 0.001).*

**Conclusion:** *There is significant increase in plasma vascular endothelial Growth Factor in ischemic stroke patients.*

### Introduction

Stroke usually referred to as cerebrovascular accident is the rapid loss of brain function due to disturbance in the blood supply to brain. WHO defines stroke as a neurological deficit of

cerebrovascular cause that persist beyond 24 hours or is interrupted by death within 24 hours. It is the second leading cause of death worldwide<sup>1</sup>.

The ischemic damage to the neural compartments is accompanied by vascular leakage, micro vessel

inflammation, and endothelial apoptosis<sup>2</sup>. Reestablishment of functional microvasculature promotes stroke recovery. During angiogenesis mature vessels are formed from vascular plexus by sprouting, branching, pruning, and differential growth of endothelial cells and recruitment of supporting cells like pericytes and smooth muscle cells<sup>3</sup>. Angiogenesis and vascular maturation are regulated by Vascular Endothelial Growth Factor (VEGF) and the angiopoietin 1/Tie 2 system<sup>4</sup>. Studies done elsewhere in stroke<sup>5,6</sup> have shown that there is elevated VEGF in patients with ischemic stroke and it can be used as a biomarker in ischemic stroke for early diagnosis and treatment. But human studies are limited in our set up. Hence the present study is carried out to compare the level of VEGF in ischemic stroke patients and asymptomatic individuals from general population.

## Aims and Objectives

### Primary Objective

To compare the level of Plasma VEGF level in patients with ischemic stroke with equal number of age and sex matched normal individuals without stroke

### Role of Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) was discovered in 1983. The VEGF gene is mapped by fluorescence in situ hybridization to chromosome 6p12.<sup>7</sup> It is one of the key proteins which induce angiogenesis in both healthy and diseased tissues.<sup>8,9,10</sup> VEGF bind to the receptors VEGF R 1 and VEGFR 2 and thus mediates intracellular signaling for cell growth. This help in the survival of ischemic cells.<sup>11</sup> It has got role in all phases of vascular development; which include;

- Vasculogenesis or production of new blood vessels from mesenchymal precursor cells<sup>12</sup>
- Angiogenesis or the hypoxia driven sprouting of new capillaries from existing vessels<sup>13</sup>

- Arteriogenesis or the enlargement of anastomotic arteriolar channels in response to blood pressure gradient<sup>14</sup>
- In addition VEGF exert direct trophic and protective effects on neurons<sup>15</sup>

Several laboratory works shows the pivotal role played by VEGF in the regulation of normal angiogenesis and abnormal angiogenesis.<sup>16</sup> Particularly the finding that the loss of even single VEGF allele results in embryonic death. This shows the irreplaceable role of VEGF in the development and differentiation of vascular structures.<sup>17,18</sup>

VEGF has potent mitogenic activity in micro vascular and macro vascular endothelial cells of arteries, veins and lymphatics. But its activity on other cell types is not consistent or appreciable<sup>19,20,21,22,23,24</sup>. In the brain VEGF is expressed normally. It is mainly expressed in the epithelial cells of choroid plexus, astrocytes, and granule cells of cerebellum.<sup>25,26</sup>

VEGF binds to endothelial tyrosine kinase receptors, they are of two types; VEGF receptor 1 (VEGFR 1 or Flt 1) and VEGF receptor 2 (VEGFR 2 or Flk 1 / KDR)<sup>27</sup>

Many studies show that hypoxia is a major inducer of VEGF mRNA expression in cells in vitro<sup>28,29</sup>. In vivo studies have shown that systemic hypoxia can induce the expression of both VEGF and VEGFR 1 in various organs including CNS.<sup>30</sup> In vitro studies show that there are three mechanisms by which biologically active VEGF is secreted by cells exposed to hypoxia.

- 1) Increased transcription rate which is mediated by the binding of HIF-1 to the 5' flanking region of the VEGF gene which code for hypoxia responsive element.<sup>31,32,33</sup>
- 2) VEGF mRNA stability is increased probably<sup>34,35</sup> by binding of the RNA binding protein HuR.<sup>36</sup>
- 3) Even under hypoxia, an internal ribosome entry site ensures efficient translation of VEGF.<sup>37</sup>

HIF 1, which is a basic helix loop helix heterodimeric transcription factor, is activated by

reduced oxygen tension. It has an alpha subunit and a beta subunit<sup>74</sup>. A homolog of HIF 1 named HIF 2 has recently been cloned<sup>38,39</sup>. It is shown to be involved in regulating VEGF gene expression<sup>40</sup>. HIF 2 has an additional role in the regulation of VEGF R<sup>241</sup>. But the involvement and activation of these two factors during cerebral ischemia is not known yet.

### Previous Studies

Research for biomarkers in ischemic stroke (REBIOS) study<sup>5</sup>. The goal of the study was to examine the temporal profile of VEGF value and its clinical significance. The subtypes of ischemic stroke were taken into consideration.

They enrolled 171 patients with ischemic stroke and age- and gender-matched healthy subjects. The stroke patients were divided into 4 subtypes: atherothrombotic infarction (ATBI, n = 34), lacunar infarction (LAC, n = 45), cardio embolic infarction (CE, n = 49) and other types (OT, n = 43). Plasma VEGF values were measured as a part of multiplex immunoassay (Human MAP v1.6) and obtained clinical information at 5 time points (days 0, 3, 7, 14 and 90) after the stroke onset.

Plasma VEGF values were significantly higher in all stroke subtypes but OT than those in the controls throughout 90 days after stroke onset. There was no significant difference in the average VEGF values among ATBI, LAC, and CE. VEGF values were positively associated with neurological severity in CE patients, while a negative association was found in ATBI patients. After adjustment for possible confounding factors, plasma VEGF value was an independent predictor of poor functional outcome in CE patients.

The study conducted by M Slevin et al<sup>6</sup>. They measured the serum levels of VEGF and active TGF- $\beta$ 1 in 29 patients with acute ischemic stroke, 26 age and sex matched individuals were used as controls. VEGF levels in serum were significantly increased in the patients after acute stroke compared with normal controls. Highest expression occurred at day 7 ( $588 \pm 121$  pg/mL; P

= 0.005), and remained significantly increased at 14 days after stroke.

Vascular endothelial growth factor expression in patients with acute ischemic stroke by Gonchar et al<sup>42</sup>. The purpose of the study was to examine the levels of vascular endothelial growth factor (VEGF) in patients with ischemic stroke (IS) in the first 48 h of disease as well as the relationship of this biomarker with the severity of neurological symptoms of stroke. They studied 44 patients with acute IS. The control group consisted of 12 healthy volunteers.

Plasma levels of VEGF were determined by ELISA. The study demonstrated the prognostic significance of VEGF for the clinical outcome of acute IS. The high level of VEGF in the first 48 h of stroke was associated with mild neurological deficit at discharge from the hospital (p=0,006). At the same time, a number of risk factors was associated with the low VEGF level at the onset of IS: extra cranial stenosis  $\geq 30\%$  (p<0,001), angina pectoris (p=0, 01) and post infarction cardio sclerosis (p=0,049).

The study conducted by Lee SC et al reported that Serum VEGF levels within 24 hours of stroke were higher in large vessel disease group than in small vessel disease group and correlate positively with infarction volume. The increase in serum VEGF levels in the acute stage resulted in improved NIHSS score after 3 months. Serum VEGF levels in the acute stage significantly correlated with the long-term prognosis of ischemic stroke<sup>43</sup>.

It could be assumed that the high concentration of VEGF is associated with the stimulation of neoangiogenesis and recovery processes that leads to a significant restoration of disturbed neurologic function and, consequently, to a favorable clinical outcome in acute cerebral infarction.

### Materials and Methods

**Study Design:** Hospital based cross sectional study

**Study Setting:** The study was done at the Department of Biochemistry and Neurology, Medical College, Thiruvananthapuram.

**Period of Study:** One year

**Sample Size:** According to the original study done at National Hospital Organization Kyushu Medical Centre Japan, calculated sample size using the formula

$$N = (Z_{\alpha} + Z_{1-\beta})^2 \times SD^2 / (\mu_1 - \mu_2)^2 = 24 \text{ Patients}$$

### Study Population

#### Inclusion Criteria

Newly diagnosed ischemic stroke patients, diagnosed by CT scan and clinical signs and symptoms in the age group 40 to 65 years admitted in The Department of Neurology. Equal number of age and sex matched individuals without stroke from general population are also included for comparison.

#### Exclusion Criteria

Patients with diabetes mellitus, rheumatoid arthritis, malignancies, pulmonary Emphysema, renal insufficiency, preeclampsia, bronchial asthma, pneumonia, severe infections and taking drugs like thiazolidinediones, bevacizumab, ranibizumab, lapatinib, sunitinib, sorafenib, axitinib, and pazopanib.

**Data Collection:** After taking informed consent, 5ml fasting blood samples drawn into plain and EDTA -Na F containing bottles from antecubital vein.

All the chemicals used for reagent preparation were of analytical grade and highest purity. Double distilled deionized water was used for reagent preparation

Plasma VEGF determined by ELISA method

For all measurements calibrators and control samples were included as precaution to quality control.

#### Estimation of VEGF

ELISA kit from BOSTER BIOLOGICAL TECHNOLOGY Co., Ltd. EL<sub>x</sub> 800MS, ERBA MICROSCAN ELISA machine.

#### Principle

Boster's human VEGF ELISA Kit is based on standard sandwich enzyme-linked immune-

sorbent assay technology. A monoclonal antibody from mouse specific for VEGF has been precoated onto 96-well plates. Standards and test samples are added to the wells; a biotinylated detection polyclonal antibody from goat specific for VEGF is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human VEGF amount of sample captured in plate.

### Analysis and Results

The mean and standard deviation for quantitative variables and percentage for qualitative variables were calculated for 24 ischemic stroke patients and 24 age and sex matched individuals from general population.

- ❖ Differences in means of quantitative variables between the two groups were compared by student t test.
- ❖ A *P* value of less than 0.05 is considered significant.

Characteristics of the participants

#### Age category

**Table 1**

Age	STROKE				Total	
	Yes		No			
	N	%	N	%	N	%
<45	8	33.3	9	37.5	17	35.4
46-55	8	33.3	7	29.2	15	31.3
>55	8	33.3	8	33.3	16	33.3
Total	24	100.0	24	100.0	48	100.0

$$\chi^2 = 0.125 \quad df = 2 \quad p = 0.939$$

There is no significant difference in the age distribution of ischemic stroke patients and the comparative group.

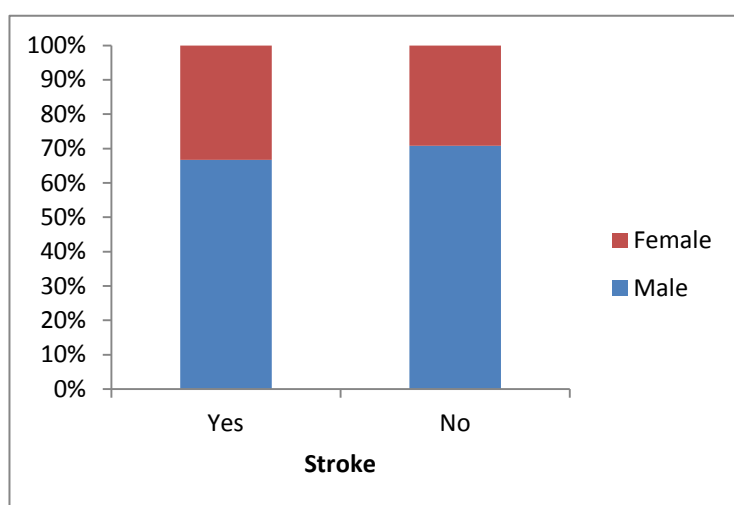
## Gender Distribution

**Table 2**

Gender	STROKE				Total	
	Yes		No			
	N	%	N	%	N	%
Male	16	66.7	17	70.8	33	68.8
Female	8	33.3	7	29.2	15	31.3
Total	24	100.0	24	100.0	48	100.0

$$\chi^2=0.097 \quad df=1 \quad p=0.755$$

In the present study there is no significant difference between the gender distribution among the ischemic stroke patients and the control group of individuals from the general population.



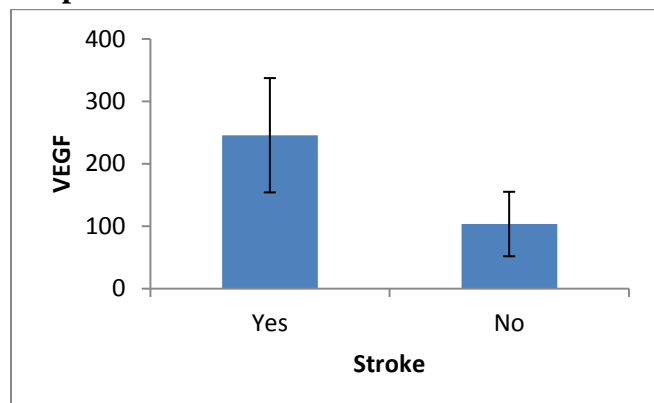
**Graph 1**

## Comparison of Plasma VEGF between Stroke Patients and Control Group

**Table 4**

Stroke	N	VEGF ( pg/ml)		T	p
		Mean	SD		
Yes	24	245.52	91.57	6.627	<0.001
No	24	103.32	51.65		

**Graph 2**



The mean VEGF (Vascular Endothelial Growth Factor) in ischemic stroke patients is 245.52pg/ml and in the comparative group is 103.32pg/ml. The difference is statistically significant with p value <0.001.

## Discussion

Stroke is one of the major causes of morbidity and mortality. Studies done elsewhere in stroke have shown that there is elevated VEGF in patients with ischemic stroke and it can be used as a biomarker in ischemic stroke for early diagnosis and treatment. But human studies are limited in our set up.

Plasma VEGF (Vascular Endothelial Growth Factor) is increased in the stroke group with mean value  $245.52 \pm 91.57$  pg / ml compared to the mean VEGF in non-stroke individuals, which is  $103.32 \pm 51.65$  pg / ml. Student t test was done to analyze the difference in mean VEGF levels between these groups and the difference was found to be statistically significant (Table-3, Graph-3) , (p value < 0.001).

Our study is comparable to the REBIOS STUDY done by Ryu Matsuo et al which shows that there is significant elevation in VEGF in ischemic stroke patients<sup>10</sup>. This study is also comparable to the study conducted by M Slevin et al<sup>11</sup>.

## Limitations of Our Study

- Our study involved smaller group of participants, so there is a need for larger studies in future.
- Our study did not assess the infarct volume among stroke patients to find the relation to severity of disease.
- As the present study was done on a particular area further studies are needed to confirm the results in other racial groups

## Future Perspectives

VEGF can be used as a biomarker for ischemic stroke.

There need to be studies to explore relation between VEGF level and infarct volume.



VEGF can be used as a prognostic indicator of ischemic stroke.

VEGF can be used as a treatment modality to decrease the severity of stroke.

### Conclusion

There is significant increase in plasma vascular endothelial Growth Factor in ischemic stroke patients.

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