A Scenario of Serum TNF α and C – Reactive Protein in Acute Ischemic Stroke and their Prognostic Significance

Zainab Yaseen, Sarita Agarwal, Manjula Shantaram, Debasish Chowdhury

Abstract

Background: Ischemic stroke comprises 85% of all stroke cases. Stroke is the third major cause of mortality worldwide and an important cause of long term disability contributing to major economic burden in most of the countries. Since inflammatory response is a key factor for ischemic damage of neurons, the markers of inflammation are supposed to have some predictive value in outcome of the ischemic stroke patients.

Objective: The purpose of this study was to estimate the biochemical markers of inflammation and atherosclerosis (CRP and TNFα) in acute ischemic stroke patients and compare them with controls. Also to correlate these parameters with stroke severity, short term outcome after 7 days and long term outcome at 6 months using modified Rankin scale and Barthel Index.

Method and Material: The present study was conducted on 45 acute ischemic stroke patients and 80 age and sex matched. The biochemical markers of inflammation and atherosclerosis (CRP and TNFα) in acute ischemic stroke patients were estimated and compared with controls. These parameters were analyzed for correlation with stroke severity (NIHSS), short term outcome after 7 days and long term outcome at 6 months using modified Rankin scale, Barthel Index and DWI MRI volume of infarct. The first blood sample was taken within 72 hours of onset of stroke and second sample was taken 7 days following the first sampling.

Results: Broadly the results showed that CRP and TNF α levels on the day of admission were higher in the experimental group as compared to controls. Difference was statistically significant. After 7 days, the levels of serum CRP and TNF α were high when compared to controls, however, the difference between day 1 and day 7 were not found to be statistically significant. When the biochemical parameters were correlated with the severity of stroke in NIHSS score, it was found that CRP on the day of admission and around 7th day correlated significantly with the severity of stroke assessed on both occasions. Correlating with the DWI MRI, volume of infarct, it was found that CRP on the day of admission as well as after 7 days correlated with the volume of infarct, therefore CRP levels correlate with the extent of tissue damage following ischemia. TNFα level did not correlate with the volume of infarct. CRP levels around 7th day also correlated with short term and long term outcome of patients assessed by modified Rankin scale and Barthel Index.

Conclusion: Measuring CRP around one week following the stroke has the greatest prognostic value.

Keywords: TNF alpha, C-reactive protein, acute ischemic stroke, serum, Barthel index, Rankin scale
SERUM TNF A AND C - REACTIVE PROTEIN IN ACUTE ISCHEMIC STROKE

INTRODUCTION
In India stroke contributes to 2% of all hospital cases and 20% of all neurological admissions. Stroke broadly includes two major types: hemorrhagic and ischemic. Of these, ischemic stroke comprises 85% of all stroke cases. The World Health Organization defined stroke as a clinical syndrome characterized by rapidly developing clinical signs and/or symptoms of focal and at times global (applied to patients with deep coma and those with subarachnoid hemorrhage) loss of cerebral functions with symptoms lasting more than 24 hours or leading to death, with no apparent cause than that of vascular origin.

Cerebral ischemia leading to infarction may be global, wherein low cerebral blood flow due to cardiac arrest or severe hypotension is maintained for longer duration or focal, when one of the vascular territories is affected either due to thrombosis and embolus. Over a past few years, a body of evidence has stressed the role of inflammation in the pathophysiology of acute brain ischemia. Inflammatory reactions which occur in acute brain ischemia are multifaceted having overlapping mechanisms and important synergistic actions with atherosclerotic processes. Sometimes, they complement the development of arterial thrombosis as well. Inflammation in atherosclerotic plaque is thought to be significant contributory factor for the plaque rupture that precedes many of the acute ischemic strokes. C-reactive protein (CRP), a sensitive indicator of systemic inflammation, has been shown to be a powerful predictor of future first-ever and recurrent coronary and cerebral ischemic event. It is also regarded as a novel marker of athero-thrombotic disease that may reflect the amount of inflammatory activity within the atherosclerotic plaque, and a direct mediator of atherogenesis. Inflammatory markers such as CRP and fibrinogen originate in liver. They are stimulated by systemic cytokines such as interleukin-1 (IL-1) β, IL 6 and tumor necrosis factor α (TNF-α). These cytokines which are intercellular signaling polypeptides are produced at extra hepatic sites such as heart, vessel walls, macrophages and adipose tissue. They are produced during acute as well as chronic inflammation as stimulators for the release of acute phase proteins. Cytokine release results in up regulation of adhesion molecules, recruitment and activation of leukocytes, promotion of leukocyte-endothelium interaction, and conversion of the local endothelium to a prothrombotic state. A few experimental and clinical studies have shown that the production of TNF-α, a cytokine is increased in acute ischemic stroke. Its prognostic significance however remains unclear. Therefore, the present study was devised to estimate these markers of inflammation and assess their prognostic significance.

MATERIALS AND METHODS
The study was conducted in the Department of Biochemistry Maulana Azad Medical College, New Delhi and Department of Neurology GB Pant Hospital, New Delhi. Prospectively consecutive acute ischemic stroke patients were included in the study. At least one and a half times number of age and sex matched normal controls were taken. Informed consent was taken from all the cases and controls. A detailed history and evaluation of risk factors were done. Baseline NIHSS (National Institute of health stroke) scale score was calculated in all patients. A standard battery of investigations (complete haemogram, Renal Function Tests, Liver Function Tests, Random Blood Sugar, lipid profile, urine routine/microscopy, X ray chest, echocardiography and carotid Doppler) was done in all the patients. Blood samples for estimation of TNFα and C- reactive protein were collected as early as possible after admission and confirmation of stroke. A second sample was taken on 7th day following the first sampling and NIHSS score was calculated on both occasions. Three measures of outcome were assessed: Recurrence of stroke or death: Clinically all patients were under a close follow up for a period of six months and any recurrence of
stroke or other vascular events and death were recorded.

**Modified Rankin Scale (MRS)**: The disability was assessed using MRS which was done on 7th day (during second sampling) and after six months.

**Barthel Index (BI)**: Functional independence was assessed using BI on 7th day and after six months.

**Inclusion criteria**
All patients of either sex aged more than 12 years presenting within 72 hours with focal neurological deficit and clinical signs consistent with WHO definition of stroke and all patients proven to have acute ischemic stroke by CT head or MRI of brain were included in the study.

**Exclusion criteria**
Patients presenting with Computed Tomography/Magnetic Resonance Imaging proven hemorrhagic stroke; patients presenting with transient ischemic attack only (deficits resolving completely within 24 hours); patients having fever at the onset or history of fever in the recent past (one week prior to stroke); patients with a history of rheumatologic diseases, autoimmune diseases, or any kind of acute or chronic infection; patients on immunosuppressive therapy, like corticosteroids, or regular analgesic uptake and patients with severe impairment of hepatic and renal functions were completely excluded from the study.

**Selection of controls**
Healthy individuals of either sex, aged more than 12 years, with no history of any major surgery or acute or chronic infection in the recent past, no history of immunosuppressive therapy, analgesic abuse or other drug abuse; no history of cardiovascular or cerebro-vascular events in the past and with no history of rheumatologic or autoimmune diseases.

The investigations for diagnosis of ischemic stroke included DWI MRI (Diffusion weighted imaging Magnetic Resonance Imaging), intracranial angiography, carotid Doppler and trans-thoracic echocardiography.

**Collection of sample**: Five ml of venous blood was collected in a clean vial after taking aseptic measures and the serum was separated as soon as possible by centrifuging at 3000 rpm for 10 minutes. The serum samples were stored in aliquots and kept at -80°C until estimation was done.

**Methods of biochemical estimations**
TNF α estimation was carried out by sandwich ELISA. C-Reactive Protein was estimated in serum using high sensitivity CRP ELISA kit provided by calbiotec Inc. (USA). It is based on the principles of solid phase enzyme linked immune sorbent assay.

The volume of the infarct was measured as per the method provided by Bisdas et al. Briefly it was as follows: Regions of interest were drawn manually around hyper-intense regions that simultaneously appeared hypodense on ADC (Apparent Diffusion Coefficient maps). The vertical and transverse diameters were measured and multiplied with 2.5 (scale provided in DWI plates.). The area obtained was multiplied with the thickness of the slice plus the gap between two slices. Each section area was added to obtain the final volume of infarct in ml. The volumes thus obtained were categorized into small (< 10 ml, medium, 11-100 ml and large > 101 ml. These were correlated with stroke severity and biochemical parameters.

**RESULTS**
CRP: The mean CRP on 1st day in the study group was 22.9±12.9 (µg/ml) and ranged from 1.6-44.4 (µg/ml). The mean CRP in control was 4.77±3.95 (µg/ml) and ranged from 0.6-14.9μg/ml. The difference was significant (p<0.0001). The mean CRP on 7th day was 23±13.17 μg/ml and was also significant when compared with the controls (Table 1).
Table 1: CRP and TNF α levels in acute ischemic stroke cases and controls

| Parameters       | Control (n=80) | Cases (n=45) | P(2 tailed) |
|------------------|----------------|--------------|-------------|
| CRP-1 (µg/ml)    |                |              |             |
| Range            | 0.6 - 14.9     | 1.6 - 44.4   | <.0001      |
| Mean±SD          | 4.77 ± 3.95    | 22.9 ± 12.9  |             |
| CRP-7 (µg/ml)    |                |              |             |
| Range            | 1.2 - 40.9     | <.0001       |
| Mean±SD          | 23.06 ±13.17   |             |             |
| TNFα-1(pg/ml)    |                |              |             |
| Range            | 0.5 - 27.7     | 1.99 - 87.2  | 0.0013      |
| Mean±SD          | 5.4 ± 4.42     | 11.85 ±16.58 |             |
| TNFα-7(pg/ml)    |                |              |             |
| Range            | 1.42 - 66.2    | 0.0003       |
| Mean±SD          | 11.86±14.28    |             |             |

CRP= C - reactive protein; TNFα = Tumor necrosis Factor alpha, SD= Standard Deviation. 2 tailed p value<0.05 is statistically significant (Mann Whitney U test)

TNFα: The mean TNFα on the 1st day in the study group was 11.85±16.58 pg./ml and ranged from 1.99-87.26 pg/ml. In the control group the mean was 5.4± 4.42 pg./ml and ranged from 0.5-27.7 pg/ml. The difference was significant. The mean on 7th day was 11.86±14.28 pg/ml and was also significant when compared with the controls (Table 1).

CRP on day 1 and day 7 significantly correlate with the severity of stroke as calculated by NIHSS score on day 1 and day 7 (Table 2). The DWI volume of infarct ranged from 0.225ml- 330ml. Mean±SD of infarct volume was 74.58±88.50 ml. 14 cases had large infarct volume >101ml, 16 cases had medium sized infarct (volume between 11-100ml) and 15 cases had small infarct (volume 0-10 ml).

Table 2: Correlation of biochemical parameters with the volume of infarct on MRI

| Parameters       | Corr. Coeff | P( 2-tailed) |
|------------------|-------------|--------------|
| CRP 1st day      | 0.463       | 0.001        |
| TNFα 1st day     | 0.128       | 0.400        |
| CRP 7th day      | 0.470       | 0.001        |
| TNFα 7th day     | -0.114      | 0.455        |

Thus CRP at the time of admission and around 7th day correlated with the volume of infarct on MRI (Table 3).

Correlation of the biochemical parameters with recurrence of stroke/TIA/death: To find out the above correlation, two groups were created in the study population: Group-1: cases with no recurrence of TIA or death; Group-2: Cases with recurrence of stroke/TIA/death within 6 months (Table 4 a) Mann Whitney U test was used. Among the two biochemical parameters CRP on 7th day post stroke predicted the recurrence of stroke/TIA or death within 6 months (Tables 4b). On correlating the biochemical parameters with modified Rankin scale and Barthel Index at 7th day and six months, it was found that CRP on day 1 and day 7 correlated with MRS and BI on day 7. However CRP on day 7 correlated with both MRS and BI even at 6 months.(Tables 5-8).

Table 3: Correlation of biochemical parameters with the volume of infarct from DWI MRI

| Parameters       | Corr. Coeff | P( 2-tailed) |
|------------------|-------------|--------------|
| CRP-1st day      | 0.5246      | 0.0002       |
| CRP-7th day      | 0.3314      | 0.026        |
| TNFα-1st day     | 0.1481      | 0.3316       |
| TNFα-7th day     | -0.0113     | 0.458        |
Table 4(a): Correlation of biochemical parameters with the recurrence of stroke /TIA (Transient Ischemic Attack) or death within 6 months

| Category      | Male  | Female |
|---------------|-------|--------|
| Group-1(n=32) | 19(59%) | 13(41%) |
| Group-2(n=13) | 7(53.8%) | 6(46.2%) |

Table 4(b): Correlation of biochemical parameters with the recurrence of stroke /TIA or death within 6 months

| Parameters*    | Group-1   | Group-2   | P (2 tailed) |
|----------------|-----------|-----------|--------------|
| CRP-1(µg/ml)  | 22.96±13.12 | 23.04±13.05 | 0.985        |
| CRP-7(µg/ml)  | 20.13±13.34 | 30.18±10.17 | 0.019        |
| TNFα-1(pg/ml) | 9.49±10.59  | 13.8±16.7  | 0.306        |
| TNFα-7(pg/ml) | 12.76±13.8  | 9.65±15.6  | 0.514        |

* - Values are mean±SD

Table 5: Correlation of biochemical parameters with Modified Rankin Scale on 7th day post stroke

| Parameters | Corr. coeff | P(2 tailed) |
|------------|-------------|-------------|
| CRP-1st day | 0.475      | 0.001       |
| CRP-7th day | 0.420      | 0.004       |
| TNF α-1st day | 0.129    | 0.395       |
| TNF α-7th day | -0.210   | 0.165       |

Table 6: Correlation of biochemical parameters with Barthel Index at 7 days post stroke

| Parameters | Corr. coeff | P(2 tailed) |
|------------|-------------|-------------|
| CRP-1st day | -0.490     | 0.001       |
| CRP-7th day | -0.449     | 0.002       |
| TNF α-1st day | 0.190    | 0.209       |
| TNF α-7th day | 0.173     | 0.255       |
DISCUSSION

Previous studies carried out on experimental animal models have shown that intraneuronal TNF α expression precedes and facilitates the entry of leucocytes to the ischemic tissue and therefore exacerbates brain damage. Moreover intra-ventricular injection of TNF α increases the infarct volume, after middle cerebral artery occlusion. From the studies on animal models it was hypothesized that TNF α level in humans following ischemic stroke should correlate with the neuro deficit and infarct volume. In the present study an increase in the levels of TNFα was found after ischemic stroke but these levels did not correlate with the severity of stroke clinically and with MRI volume of infarct nor did they correlate with the outcome. Our findings are similar to those described by Intiso et al who reported that the levels of TNF α after ischemic stroke do not correlate with the infarct size. In another study by Ferrarese et al, TNF α levels increased in peripheral blood from 24 hours of stroke onset up to 90 days without any peak. The levels however did not show correlations with the infarct volume or stroke severity on NIHSS scale. But the findings in different studies are not consistent. In one study by Losy et al TNF α levels were shown to increase within 24 hours of stroke onset and the peak levels correlated with clinical stroke severity and inversely correlated with the Barthel Index after 1 week. Castillo et al conducted a case-control study with 231 cases of acute ischemic stroke and found that the levels of TNF α were higher in cases with early neurological deterioration, but the relationship was confounded by other factors and did not remain significant on multivariate testing.

However, it should be emphasized that the inflammatory reactions are dual edged swords and the cytokines can act both as killers and healers. Previous experimental studies on animal models have shown that TNFα receptor knock-out mice had larger infarct size. In the present study Interestingly, the correlation coefficient of TNF α on 7th day with NIHSS score, DWI volume of infarct and MRS was negative and that with Barthel index was positive. This means that the patients with higher TNF α on day 7 had less severe NIHSS score, lesser volume of infarct in DWI and better outcome at 6 months on MRS and BI. This might be due to complex interplay between neuronal damaging role of TNF α in the acute phase of stroke in contrast to the neuro-protective role of TNF α once the acute phase of stroke is over.

| Parameters | Corr coeff | P(2 tailed) |
|------------|------------|-------------|
| CRP-1      | 0.283      | 0.059       |
| CRP-7      | 0.458      | 0.001       |
| TNF α-1    | -0.028     | 0.853       |
| TNF α-7    | -0.074     | 0.626       |

Table 7: Correlation of biochemical parameters with Modified Rankin Scale at 6 months

| Parameters | Corr coeff | P(2 tailed) |
|------------|------------|-------------|
| CRP-1      | -0.201     | 0.185       |
| CRP-7      | -0.501     | 0.0005      |
| TNF α-1    | 0.55       | 0.719       |
| TNF α-7    | 0.120      | 0.431       |

Table 8: Correlation of biochemical parameters with Barthel Index at 6 months
In our study, the CRP levels are higher in stroke patients at the time of admission and increased further after a week following the ictus as compared to controls. It is well known that CRP is a marker of atherosclerosis. Moreover, CRP gets elevated in response to ischemic tissue damage. The rising trend seen in our study supports these facts as the rise can be explained on the basis of ischemic tissue damage upon a background of atherosclerotic process going on in these patients. Previous studies have also demonstrated a rise in CRP levels within 24 hours of stroke which peaks at 48-72 hours and the levels remain elevated up to 3 months.

CRP is an acute phase reactant and several prospective studies have demonstrated that a single non-fasting measurement of CRP is a predictor of future fatal and nonfatal cerebrovascular events. In the acute phase of stroke, inflammation contributes to brain damage initiated by ischemia. There is release of cytokines, adhesion molecules and acute phase proteins. The hypothesis that high concentrations of CRP in acute phase of stroke could reflect the extent and severity of cerebral injury was tested in our study. It was found that CRP levels on the day of admission and after 7 days correlated strongly with the severity of stroke as assessed by NIHSS score. Similar findings were reported by Smith et al. They found a strong correlation between CRP levels within 24 hours of stroke and NIHSS score. The levels of CRP on day of admission and at 7 days correlated with the MRI volume of infarct, but CRP on day 1 showed better correlation than that on day 7. Iyigun et al also found a significant correlation between CRP level measured within 72 hours of stroke onset and size of infarct. However Smith et al found better correlation of infarct volume with CRP levels at first week following stroke. The authors however did not mention the time of imaging following stroke. The mean time of imaging following stroke in our study was 66.2 hours. The elevated CRP levels on 7th day were also related to the recurrence of stroke or death within 6 months in our study. Most of the previous studies have emphasized the importance of CRP levels within 12-24 hours of stroke in predicting the clinical outcome; however in one study by Iyigun et al the CRP levels at discharge /1st week was found to have better predictive value in clinical outcome. Massotti et al in 2005, also reported the importance of CRP levels at discharge in predicting future vascular events and death. In our study, when the CRP levels were correlated with the disability outcome on MRS and functional outcome on BI, it was found that CRP levels on the day of admission correlated with the disability and outcome on 7th day but the predictive value did not extend up to 6 months. However CRP levels after 7 days had a stronger predictive value after 6 months. These findings corroborates with those described by Iyigun et al, and Masotti et al, who also found that CRP levels at discharge are better predictors of outcome. However Silvestri et al have reported in their study that the CRP levels at discharge did not predict outcome after one year. In our study, the patients were followed up only up to 6 months hence we cannot comment on the predictive values of CRP estimation after one year.

Limitations of the study

The sample size was small, compared to other studies. The timing of first sample was within 72 hours, which was slightly longer than other studies, but time was chosen on logic grounds. However, on sub-analysis of this time window, much difference was not found in the value of the biochemical parameters except for CRP which was higher in the 48-72 hours subgroup. MRI volume was measured using semi-automated technique, which is slightly less accurate than the fully automated techniques; however it was felt that this did not affect the results.

Conclusion

C-reactive protein and TNF-α levels rise in the acute phase following ischemic stroke. The levels of CRP and TNF-α increased by 7 days. The levels of CRP on the day of admission and after 7 days correlate positively with the severity of stroke as
SERUM TNF Α AND C - REACTIVE PROTEIN IN ACUTE ISCHEMIC STROKE

measured by NIHSS (national institute of health stroke scale) score. The levels of CRP on the day of admission and after 7 days correlate positively with the DWI MRI volume of infarct. CRP on the day of admission correlate with the short term prognosis of the patients.(measured by MRS and BI).CRP levels on 7th day correlates with long term prognosis of the patients. TNF α levels do not have any correlation with the outcome of the patients.

**Ethical Clearance:** We certify that all applicable institutional regulations concerning the ethical use of human volunteers were followed during the course of research

**REFERENCES**

1. Stroke in Park’s Text book of Preventive and Social Medicine.18th Edition. K Park(Ed) Jabalpur. India. Banarasidas Bhanot 2004.p 298-299.

2. Wade S Smith, Johnston Sc, Donald Ej. Cerebrovascular diseases in Harrisons principles of Internal Medicine (vol 2) 15th ed, Braunwald Fauci, Kasper Hauser, Longo, Jameson(eds).North America. Mac Grawhill. 2004.p 2369-2391.

3. Hatano S. Variability of diagnosis of stroke by clinical judgement and by a scoring method. Bull World Health Organ 1976; 54: 533-540.

4. Becker KJ. Inflammation and acute stroke. Current opinion in neurology.1998; 11:45-49.

5. Ross R. Atherosclerosis: an inflammatory disease. N Engl J of Med 1999; 340:115-126.

6. Ramadori G, Christ B.Cytokines and the hepatic acute phase. Semin Liver Dis 1999;19: 141-155.

7. Arvin B, Nivelle LF, Barone FC, Feurestein GZ. The role of inflammation and cytokines in brain injury.Neuro Sci BiobehavRev 1996;20: 445-452.

8. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: New opportunities for novel therapies. J Cereb Blood flow Metab 1999; 19: 819-834.

9. Hallenback JM. The many faces of Tumor necrosis factor in Stroke. Nature Med 2002; 8: 1363-1368.

10. Hopkins SJ, Rothwell NJ. Cytokines and the nervous system Expression and recognition. Trends Neuro ci 1995; 18: 130-136.

11. Hopkins SJ, Rothwell NJ. Cytokines and the nervous system II actions and mechanism of actions. Trends Neuro Sci 2000; 23: 618-625.

12. Wilson JTL, Harendran A, Potter J, Bone I, Muir K W. Reliability of the modified Rankin Scale across Multiple Raters Benefits of a Structured Interview. Stroke 2005;36: 777-781.

13. Mahoney Florence I Barthel: Functional Evaluation: The Barthel Index. Maryland State Medical Journal 1965; Feb: 61-63.

14. Hedayati M, Yazdanparast R, Azizi F.Determination of human tumor necrosis factor alpha by a highly sensitive enzyme immunoassay.Biochem Biophys Res Comm 2001; Nov: 23:289(1):295-298.

15. Yuan CS. Methods and composition of assaying analytes. U.S Patent No. US 6,376, 210B1 (202).

16. Bisdas S, Donnerstag F, Ahl B, Bohrer I, Weinsenborn K, Becker H. Comparison of perfusion computed tomography with diffusion weighted magnetic resonance imaging in hyperacute ischemic stroke. J. Comput. Assist. Tomogr 2004; 28(6): 747-755.

17. Siren AL, Eliahu H, David D, et al. Release of proinflammatory and prothrombotic mediators in the brain and peripheral circulation in spontaneously hypertensive and normotensive wistar Kyoto rats. Stroke 1992; 23: 1643-1651.

18. Barone FC, Arvin B, White RF, et al. Tumor necrosis factor α, a mediator of focal ischemic brain injury. Stroke 1997; 28: 1233-1244.

19. Kochanek PM, Hallenbeck JM. Polymorphonuclear leucocytes and monocyte/macrophages in the pathogenesis of cerebral ischemia and stroke. Stroke 1992; 23: 1367-1379.

20. Intiso D, Zarelli MM, Lagioia G, et al. Tumor Necrosis Factor Alpha Serum levels and inflammatory response in acute ischemic stroke patients. Neurosciences 2000; 24: 390-396.

21. Ferrarese C, Masaruacci P, Zoia C, et al. Increased cytokine release from peripheral blood cells after acute stroke. J Cereb Blood flow Metab 1999; 19: 1004-1009.
22. Zaremba J, Losy J. Early TNF –α levels correlate with ischemic stroke severity. Acta Neurol Scan 2001;104: 288-295.
23. Castillo J, Ira R. Biochemical changes and inflammatory response as markers for brain ischemia: molecular markers of diagnostic utility and prognosis in human clinical practice. Cerebrovas dis 204; 17(suppl 1):7-18.
24. Bruce A J, Boling W, Kindy M S, et al. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nature Med 1996; 2: 788-794.
25. Muir KW, Weir CJ, Alwan W, Squire IB, Lees KR. C-reactive protein and outcome after ischemic stroke. Stroke 1999; 30:981-985.
26. Di Napoli M, Papa F, Bocola V. Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. Stroke 2001; 32: 133-138.
27. Christensen H, Boysen G. C-reactive protein and white blood cell count increases in the first 24 hours after acute stroke. Cerebrovascular diseases 2004; 18:214-219.
28. Di Napoli M. C-reactive protein and acute phase of ischemic stroke. BMJ 2001;322: 1605-1606.
29. Di Napoli M, Farncesca P, Bocola V. C-reactive protein in ischemic stroke. An independent prognostic factor. Stroke 2001; 32: 917-924.
30. Masotti L, Cecearelli E, Forconi S, Capelli R. Prognostic role of C-reactive protein in very old patients with acute ischemic stroke. Journal of Internal Medicine 2005; 258: 145-152.
31. Di Napoli M, Markus S, Roberto C, et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke. A statement for Health care professionals from the CRP pooling project members. Stroke 2005; 36: 1316-1329.
32. Di Napoli M. Early inflammatory response in ischemic stroke. Thromb Res 2001; 103:261-264.
33. Smith CJ. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischemic stroke correlate with brain infarct volume, stroke severity and long term outcome. BMC Neurology 2004; 4:2.
34. Iyigun I, Bakirei Y. Plasma Concentrations of C-reactive protein and Fibrinogen in Ischemic Stroke. J Int Med Res. 2002;30: 591-596.
35. Silvestri A, Vitale C, Ferretti F, Onorati D, Fini M, Rosano GM. Plasma levels of C-reactive protein and interleukin -6 predict outcome in elderly patients with stroke. J Am Geriatric Soc 2004; 52: 1586-1587.