# Change in tissue thromboplastin content of brain following trauma

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Background: Tissue thromboplastin (TTP) is an integral membrane protein contributing to coagulopathy after trauma of brain, which is a rich source of TTP. Aims: A study was undertaken to establish the TTP content of various areas of normal brain and estimate the changes in TTP activity of brain in response to varying degrees of trauma. Materials and Methods: Samples from different areas of brain of ten cadavers were used as controls and they were compared with contused brain tissue obtained after surgery in 25 head injury (HI) patients of varying severity. Results: In the study group, the TTP activity of the frontal, parietal, and temporal lobes after HI was significantly raised in contrast to that of the control group. The TTP activity was also significantly higher in the severe HI patients than those having moderate HI. The mode of injury and the time lapse after HI had no significant bearing on the TTP activity. Subjects above 40 years of age demonstrated a higher mean TTP activity after HI, though it was not statistically significant. Conclusion: The study provides quantitative data on TTP activity of normal brain and highlights the role of TTP in coagulopathy following HI through its increased activity after HI, more so in the severe HI group.

Key Words: Head injury, tissue thromboplastin, tissue factor

Tissue thromboplastin (TTP), also called tissue factor (TF), plays a pivotal role in the coagulation cascade. It is considered as the physiological initiator of coagulation when it comes in contact with the blood at the site of an injury. Tissue thromboplastin catalyses the conversion of prothrombin to thrombin in the presence of  $Ca^{2+}$  in the initiation of blood coagulation.

Tissue thromboplastin activity is found in extracts of almost all tissues of the body, human brain being a rich source. The comparison of activities of TTP from 34 different areas of brain by Bjorkud *et al.* demonstrated a characteristic distribution pattern and a wide range of activities.<sup>[1]</sup> However, there is paucity in the literature on studies that give a quantitative measurement of TTP activity of other organs in their normal or pathological state.

Head injury (HI) is a common surgical emergency encountered in this fast-paced modern life. Coagulation disorders including disseminated intravascular coagulation (DIC) are well-known complications of HI recognized for decades.<sup>[2:4]</sup> Delayed brain changes following HI-like cerebral contusion or haematoma seem to appear as fresh progressive lesions in computerized tomography (CT) scans done at different intervals,<sup>[5]</sup> and sometimes patients of HI develop unexplained postoperative haematoma.

As several factors may be operating in the pathophysiology of post-HI-coagulation disorders including DIC, delayed cerebral contusion or postoperative cerebral haematoma, it stands to logic that this phenomenon might have something to do with the tissue thromboplastic content released as a result of trauma to the brain tissue, which is a known rich source of TTP. Though the TTP content of the brain has been estimated in normal individuals in one study till date,<sup>[1]</sup> the literature is lacking in data on alteration of the TTP content of various areas of the brain in response to trauma.

The present study was aimed to: (1) establish the TTP content of various areas of normal brain, (2) estimate the changes in TTP content of various areas of brain in response to trauma, (3) establish any correlation of TTP levels in the brain vis-àvis severity of HI.

# Materials and Methods

The present study was conducted over a 2-year time frame with the approval of the Institute Research Committee, who looked into the ethical considerations of the study before giving the approval.

# Control group

The control group comprised ten cadavers of patients who died of conditions not affecting normal coagulation. The brain tissues were

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harvested within 6 h of death during medicolegal postmortem examination in the Department of forensic medicine. Tissue samples from different areas of brain were collected with the valid consent of legal heirs of the deceased. Brain tissue samples were collected from the following areas: (a) gray and white matter of all lobes, (b) thalamus, (c) optic nerve and tract, (d) pons, (e) medulla oblongata, (f) cerebellum, and (g) mid brain.

## Study group (head-injury patients)

Twenty-five patients with HI admitted under the neurosurgical services of the Institute were included in the study. All the cases underwent surgery after admission once they were found to have a normal screening coagulation profile.

There were 22 male and 3 female patients of which 19 (76%) patients sustained HI in roadside accidents, and 6 (24%) patients had a history of fall from height. Age ranged between 14 and 70 years (mean 42.96  $\pm$  18.84), with 15 patients below 50 years of age, 5 in the fifth decade, and the remaining 5 above 60 years of age.

Location of contusion of the brains was classified according to the predominant lobe involved. Patients were divided into three groups according to the severity of injury using the Glasgow Coma Scale (GCS): Minor HI (Group A: GCS 13-15), moderate HI (Group B: GCS 9-12), and severe HI (Group C: CGS 3-8). Accordingly, 2 (8%) patients fell in Group A, 11 (44%) in Group B, and 12 (48%) in Group C at the time of admission. All patients underwent detailed neurological examination. The level of consciousness varied from patient to patient. Twelve patients were put on assisted ventilation before or after admission.

### CT scan findings

A CT scan revealed cerebral contusion involving one or more than one lobe in all the patients. Four patients had associated skull fracture, two had associated SDH, one had SAH and intraventricular hemorrhage and the remaining one had associated EDH. Eleven (44%) patients had predominantly frontal lobe contusion followed by temporal lobe contusion in six (24%) and parietal lobe contusion in eight (32%). No patient had contusion of the occipital lobe.

Nineteen (76%) patients were operated on within 8-24 h of admission. The rest were operated on late due to delay in admission or late deterioration. All patients underwent craniotomy and evacuation of contused brain tissue, which was collected for estimation of TTP activity after washing with saline to remove blood clots.

#### Collection and processing of brain tissue

Contused brain tissue samples from patients and normal brain tissue samples from the control group were collected in sterile saline immediately after resection in the operation theatre or autopsy room as per the case. The specimens were delivered to the laboratory within 15 min of collection. If that was not feasible, the collected specimen was preserved in autoclaved normal saline in a refrigerator at 4°C. Each tissue was washed several times with normal saline to remove blood and other tissue fluids, minced, and then homogenized in a tissue homogenizer using a tight pestle. The homogenate was centrifuged at 8000 g for 30 min and nonhomogenized tissue debris was discarded. The protein content of the supernatant was estimated by Lowry's method<sup>[6]</sup> and the sample was stored at -20°C until further analysis.

The tissue homogenate (TH) was thawed immediately before use and diluted in normal saline to a final protein concentration of 1 mg/

ml-1 before use in coagulation studies.<sup>[7]</sup>

#### Assay of brain TTP activity

Plasma recalcification time was noted after addition of 0.1 ml of TH to 0.1 ml of normal pooled plasma. This was compared with a standard TTP calibration curve. Standard graph was plotted using commercially available recombinant human thromboplastin. The clotting time obtained with 1 mg/ml protein concentration of recombinant human thromboplastin was taken to represent 100% TTP activity. Contused brain TTP was thus expressed as percentage activity of recombinant human TTP.

The TTP activity of different areas of normal brain was tabulated and the mean TTP value of individual areas of normal brain was established. The TTP activity of contused or traumatized brain tissue collected from patients of HI was estimated and compared with the corresponding mean value of control subjects. The results were analyzed subsequently after tabulation using unpaired t-test.

As ours is a tertiary referral center, all the patients were discharged for convalescence and further management to the local hospital. However, due to various logistic problems a satisfactory data on the follow up of these patients could not be obtained.

## Results

#### Control group

The TTP activity of different areas of the brain was estimated in ten cadavers as control, which is depicted in Table 1. The TTP activity from different areas of the brains of ten cadavers was studied as per inclusion criteria for control group and TTP activity was estimated as per description in the methodology. The overall mean TTP activity of control group was  $75.084\% \pm 29.6$  (range 25-146%). The control samples were divided into two groups - lobar and nonlobar samples. The lobar samples consisted of brain tissue from frontal, temporal, parietal, and occipital lobes. The nonlobar group consisted of optic nerve, medulla oblongata, pons, mid brain, thalamus, and cerebellum. In the lobar control group, mean TTP activity was  $83.85\% \pm 31.29$  (range 27-146%) while in nonlobar group, it was  $70.63 \pm 27.76$  (range 25%-136%). In the nonlobar group, the highest mean TTP activity was seen in pons (136%) and lowest activity in mid brain (25%). Comparing the lobar control samples and nonlobar control samples, the unpaired 't' test was found to be statistically significant (t = 2.216; P=0.029).

Table 1: Mean tissue thromboplastin activity of different areas of the brain (control group)		
Area	Range (%)	Mean ± SD (%)
Frontal lobe	65-110	95% ± 21.21
Temporal lobe	55-146	100.4% ± 36.11
Parietal lobe	27-105	60.6% ± 32.72
Occipital lobe	50-100	97.4% ± 18.35
Cerebellum	69-86	78.8% ± 5.8
Optic nerve	27-65	47.2% ± 15.63
Medulla	40-86	64% ± 18.35
Pons	100-130	114.6% ± 13.6
Mid brain	25-50	39.2% ± 10.53
Thalamus	70-90	80% ± 8.16

# TTP activity of study group

The TTP activity from brain tissue of 25 HI patients was estimated. The mean value of TTP activity of the study group was  $164.76\% \pm 72.78$  (range 82.350%). The frontal lobe was the most common site (44%) for lobar contusion. This was followed by temporal (32%) and parietal lobes (24%), respectively. No patient had occipital lobe contusion. In 8 (32%) patients, the TTP activity was above 200%, while in 14 (56%) patients, TTP activity ranged between 100 and 200%. The remaining three (12%) patients had TTP activity below 100% but greater than 50%. The mean TTP activity as per different lobes affected in the study group is depicted in Table 2, with parietal lobe showing the highest activity.

Comparison of the TTP activity of corresponding lobes of both control and study groups show that the overall TTP activity in the study group is significantly raised in comparison to the control group  $(t = 5.3; P \le 0.001)$ .

Twelve of twenty-five cases that belonged to the severe HI group had TTP activity that ranged between 110 and 350% (mean value of 204.1 ± 78.83). Eleven patients with moderate HI had TTP activity ranging between 94 and 240% (mean 136% ± 43.56). Two patients suffered mild HI [Figure 1]. The comparison between moderate and severe HI groups established a statistically significant difference in TTP activity (t = 2.53; P = 0.019). The mean TTP activity of the severe HI group was higher than the mild HI group but it failed to reach a statistical significance.

Tissue thromboplastin activity of different lobes of normal brain was compared with corresponding lobes with contused brain tissue. The mean TTP activity of contused brain from three lobes (frontal, temporal, and parietal) was higher than that of normal brain. This difference was statistically significant for all the lobes (frontal lobe t = 3.02, P = 0.007; parietal lobe t = 3.73, P = 0.002, temporal lobe t = 2.26, P = 0.038).

Tissue thromboplastin activity versus mode of injury was correlated between two groups where HI was sustained by (i) fall from height (six patients) and (ii) roadside accident (19 patients). The mean TTP activity of the former group was  $156.67 \pm 79.63$  (range 82-300%) and the latter was  $167.32 \pm 72.61$  (range 92-350%). The difference was not statistically significant (t = 0.3; P = 0.76).

Tissue thromboplastin activity was correlated with the interval between the time of injury and surgery. The patients were divided between two groups where surgery was performed (1) within 8-24 h and (2) those operated after 24 h. The

Table 2: Mean tissue thromboplastin (TTP) activity of different lobes in the study group			
Lobe	Range (%)	Mean ± SD (%)	
Frontal	82-240	161% ± 65.92	
Temporal	94-240	149.75% ± 56.32	
Parietal	111-350	191.67 ± 105.04	



Figure1: Tissue thromboplastin activity in patients with head injury according to the severity of injury

mean TTP activity of patients operated after 24 h was 162.17  $\pm$  54.12 and of those operated within 8-24 h 165.58  $\pm$  79.03; the correlation was not statistically significant (t = 0.098; P = 0.92).

On correlation of the TTP activity of contused brain tissue with the age of patients, it was found that patients above 40 years of age showed a higher mean TTP activity (mean  $182 \pm 63.4$ ; range 110-300%) than those below 40 years of age (mean 142.82  $\pm$  80.87; range 82-350%). The correlation, however, failed to reach a statistical significance (t =1.36; P = 0.18).

# Discussion

Since the description of the cascade system of coagulation for the first time in 1964, it has long been recognized that with an injured subendothelium the surface of adherent platelets plays an essential role in initiating and supporting coagulation reactions. Early observation on blood clotting established the potent ability of certain tissue extracts, notably from brain and lung, to initiate fibrin formation. These tissue extracts (thromboplastins) were found to contain two ingredients responsible for this activity – a phospholipid component and a protein component. This led to the discovery of tissue factor (TF), an integral membrane protein expressed by cells in most extra-vascular tissues. Tissue factor is not present in the plasma but is supplied by exogenous tissue. It is known that TF is not always extrinsic to the circulatory system but is expressed on the surface of vascular endothelial cells and leucocytes under certain pathological conditions, whereby it is thought to contribute to associated coagulopathy.<sup>[8]</sup>

Comparison of the TTP activity of different areas of normal brain demonstrated a wide range of variations.<sup>[1]</sup> It was also noted that the distinct distribution pattern was unrelated to tissue vascularization and white and gray matter had similar activities. The highest activity was observed in olfactory bulb and tract. Regions associated with the limbic system, second cranial nerve and primary visual structures and lower part of the brain stem also have a very high-procoagulant activity. High TTP activity seemed to occur in phylogenetically older parts of the brain.

The first case with definite evidence of DIC associated with a massive HI was reported by Keimowitz and Annis in 1973.<sup>[9]</sup> Vander Sande *et al*<sup>[10]</sup> described the relationship of CT and coagulation abnormalities to clinical features in 55 patients with blunt HI. They concluded that (a) FDP concentration reflected the amount of brain tissue damage rather than its location, (b) in the absence of other causes of DIC, coagulation studies may be more sensitive than CT scanning in demonstrating brain contusion. It was interpreted that the finding of high FDP with a combination of hemorrhagic lesion in the brain and mass effect was a confirmation of hypothesized infusion of TTP into the circulation consequent to head trauma.

Scherer *et al*<sup>[11]</sup> tried to determine the degree of regional and systemic coagulation activation soon after isolated severe HI. They studied 24 trauma victims, of which 20 patients had isolated severe HI (GCS  $\leq 8$ ) and four patients with isolated bone fractures. It was concluded that within 6 h after severe isolated HI, systemic procoagulant overflow from the traumatized cerebral microvasculature proceeds to thrombin level and is then inhibited by antithrombin III. Regional and systemic hypercoagulability and increased D-dimer concentrations appear to be common among HI patients. Increased procoagulant and consecutive fibrinolytic turnover may, therefore, spark DIC in this patient group.

Various earlier studies established coagulation abnormalities in HI<sup>[2-4]</sup> leading to DIC also.<sup>[9,12-14]</sup> All these studies highlighted the role of TTP in the genesis of coagulation abnormalities in HI. The present study is a step ahead to establish the above postulate and prove the role of TTP in the causation of coagulation disorders after HI. There is a statistically significant correlation of TTP activity between moderate and severe HI. Hirashima et al. tried to determine whether TF might be a marker of brain injury caused by SAH and cerebral vasospasm.<sup>[15]</sup> Using a sensitive enzyme immunoassay, the authors measured the TF in CSF of patients with SAH. As the concentration of TF in CSF was increased fivefold in comparison to plasma after the onset of SAH, it was believed that TF was released from the brain with its concentration paralleling the degree of tissue injury. Hence, the release of TF after brain injury may initiate coagulation disorders. Drawing a parallel example in HI, which is very frequently associated with SAH, evidence from present study establishes the role of TTP activity as a marker of HI. The high level of TTP activity correlated with the severity of HI. The mechanism of release of TTP after HI is yet to be established. Postulated mechanisms suggest that there may be increased synthesis or there is an enhanced release of TTP following trauma or surface expression of TTP activity already present in the

contused brain tissue. However, further research needs to be done in this direction.

Robert *et al.* described the development of a mutant of human TF as a specific antagonist of the extrinsic pathway of blood coagulation and tested this mutant in a rabbit model of arterial thrombosis.<sup>[16]</sup>

Creasey *et al.* (1993) showed the role of tissue factor pathway inhibitor (TFPI) in reducing mortality from *Escherichia coli* and septic shock.<sup>[17]</sup> This fact leads to the assumption of the role of an analogue of TFPI in the management of moderate to severe HI patients, who are at a greater risk of coagulation disorder or DIC.

The present study not only attempts to establish a quantitative data on basal TTP activity in different areas of normal brain tissue but also establishes the role of TTP in the promotion of coagulation disorders in HI patients, with its potential to act as a prognostic indicator. On the whole, the study highlighted the role of TTP activity in severe HI, as there is significant difference in TTP activity of this group from the moderate HI patients.

The patients of HI were taken up for surgery after they had a normal screening coagulation profile but postoperative screening coagulation tests were not included in the data as these were bound to have secondary alterations by the insult of surgery on the brain and blood transfusion, which was required in some cases. However, there remains a scope for future study using more sensitive and specific activation markers of coagulation to establish any correlation between coagulation changes and TTP activity of the brain tissue.

The present study is the first of its kind in which the TTP activity of brain parenchyma after HI has been quantified, as earlier studies have used only the hematological changes subsequent to head trauma as a parameter to prognosticate HI.<sup>[18-20]</sup> This might have some implications in the management of HI in future. However, further studies are required on a larger number of patients to endorse the above findings. Patients with moderate to severe HI with evidence of coagulation disturbances may at a later date benefit from adjunctive therapy with mutant TF or with TFPI.

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