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Tenascin takes part in the progress of pathological severity in cerebral falciparum infection

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We examined plasma level of circulating form of tenascin (TC) in falciparum malaria patients, cerebral malaria and non cerebral malaria, and compared them to uninfected healthy persons. Plasma level of TC examined were significantly higher in malaria patients than in control persons ($p< 0.01$). The results also show that among malaria patients, TC level was higher in the cerebral malaria patients compare to non cerebral malaria patients ($p<0.10$). Kinetics of TC level in plasma were related to kinetics of TNF-*a* level. Moreover, patients with higher level of TC showed higher level of parasitized erythrocytes binding ratio. Immunohistochemical study showed that TC was found in the cerebral microvessels of postmortem cerebral tissues from nine cerebral malaria cases. These results provide evidence that plasma level of TC correlates with the severity in cerebral malaria patients.

Keywords : Tenascin, *Plasmodium falciparum*, Cerebral malaria, TNF-*a*, Extracellular matrix protein

INTRODUCTION

Tenascin (TC) is the most unusual of the extra cellular matrix proteins, and is a disulfide-linked hexameric extracellular-matrix protein with three subunits. TC is containing cell binding domain such as RGD and IRVVM that also contain in thrombospondin [1]. Recently, the presence of TC has been demonstrated in normal human sera and elevated serum TC levels were shown in melanoma patients and various kind of cancer patients [1, 4-5]. It has been suggested that TC may serve as tumor marker. TC expression in the tissues also elevated in above diseases and inflammatory conditions of the urinary bladder. Schenk *et al.* [4] reported that serum level of TC in malaria patient was higher than control. However, no similar study has been found. In this study, we examined plasma level of circulating form of TC in falciparum malaria patients, cerebral malaria and non cerebral malaria, and compared them to uninfected healthy persons. Moreover, we investigate the relations between plasma level of TC and laboratory and parasitological findings.

MATERIALS AND METHODS

Plasma samples were obtained from malaria patients admitted to the hospital in Myanmar and Thailand at the admission day. They had various parasitemia levels and a variety of cerebral disturbance. Plasma samples were also obtained from Japanese volunteers as the control who had no experience of malaria. Postmortem brain tissues were collected from nine severe falciparum malaria cases.

Plasma TC levels were analyzed with a double antibody sandwich enzyme-linked immunosorbent assay using a MAb (7-13) as

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capture antibody and a MAb (36-13-6) as a detection antibody. Flat-bottomed 96-well microplates were coated with 50 μl of MAb 7-13 (10 μg/ml) and blocked with 3% BSA. After the blocking, 50 μl of plasma samples were added in each well and incubated. The microplates were washed three times. After incubated with 50 μl biotinylated MAb 36-13-6 (2 μg/ml), the plates were washed again and incubated with peroxidase labeled avidin (1:1000). The plates were then washed three times. The enzyme reaction was carried out by adding 100 μl of enzyme substrate (4mM 1,2-phenylenediamine, 0.003% hydrogen peroxide in 0.1M acetate buffer, pH 6.0) The reaction was stopped with 50 μl of 2N sulfuric acid and read at 490 nm.

For immunohistochemical study, brain tissues were fixed in 10% buffered formalin, embedded in paraffin. To examine TC, an avidin-biotin peroxidase complex method was applied to the paraffin-embedded brain tissues. The endogenous peroxidase activity was blocked by 2% hydrogen peroxide in 60% methanol. Nonspecific binding was blocked with normal horse serum in PBS. The sections then were incubated with MAb 7-13 (10 μg/ml). After rinsing, the sections were incubated with biotinylated anti mouse IgG. The sections then rinsed and incubated with ABC complex. The color was developed with diaminobenzidine tetrahydrochloride (0.1 mg/ml in 0.05M Tris buffer containing 0.2 μl of hydrogen peroxide) using the ABC complex as the substrate. As the negative control, brain sections from malaria-negative accidental dead patients that were collected at Fujita Health University Hospital were tested for the presence of TC.

RESULTS AND DISCUSSION

We have investigated plasma TC levels in 37 patients infected with *P. falciparum*. As a control we measured TC levels in plasma of ten healthy persons. Falciparum malaria patients have significantly higher TC levels than the normal control group ($p<0.01$) (Fig. 1). The results also showed that among falciparum malaria patients, TC levels were higher in the cerebral malaria patients compared to non cerebral malaria patients ($p<0.10$) (Fig. 2).

The seven patients were followed up for one month, with plasma samples being taken at five occasions (day 0, 7, 14, 21, and 28). After admission day, mean value of plasma TC level was once increased then reduced. On day seven, it was increased to the peak level during day zero to day 28. On day 28, it was reduced to the minimum level that was approximately one half of the level at the day zero.

Schenk *et al.* [4] reported that serum level of TC in the cancer patients was correlation

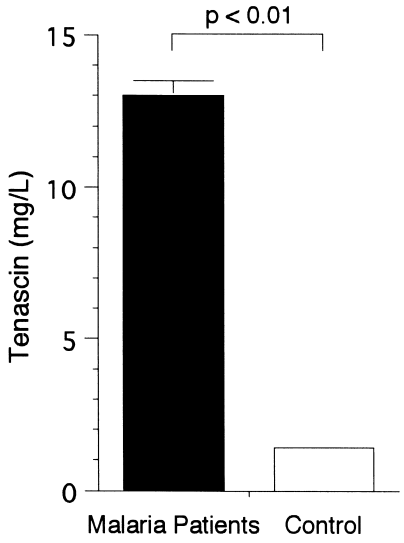

Fig. 1 Plasma level of tenascin in falciparum malaria patients and healthy persons.

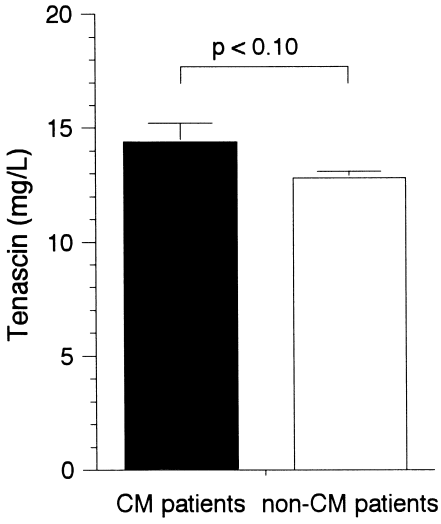

Fig. 2 Comparison of plasma tenascin level between cerebral malaria patients and non cerebral malaria patients.

to CRP (C-reactive protein). The result in this study showed that plasma level of TC in the falciparum patients was not significantly correlated to CRP. Moreover, we analyzed relationships between plasma level of TC and parasitological and laboratory findings for *in vivo* function of TC. The results also showed that plasma level of TC was significantly correlated to TNF-α level in the plasma (Fig. 3), although plasma level of TC had no correlation to anti-*P. falciparum* antibody titer, parasitemia, and the infection over last two years. Schenk *et al*. [3] reported similar data that TNF and IFN-γ induced a rapid increase in plasma TC levels.

As TC functioned as adhesion substrate [1], we analyzed relationships between the binding ratio to C32 melanoma cells of the parasitized erythrocytes that were collected at the admission day. As the result, patients with higher level of TC showed higher level of parasitized erythrocytes binding ratio (Fig. 4).

Immunohistochemical study showed that TC was found in the cerebral microvessels of postmortem cerebral tissues from nine cerebral malaria cases, although control brain tissues were negative.

These results provide evidence that plasma level of TC correlates with the severity in cerebral malaria patients.

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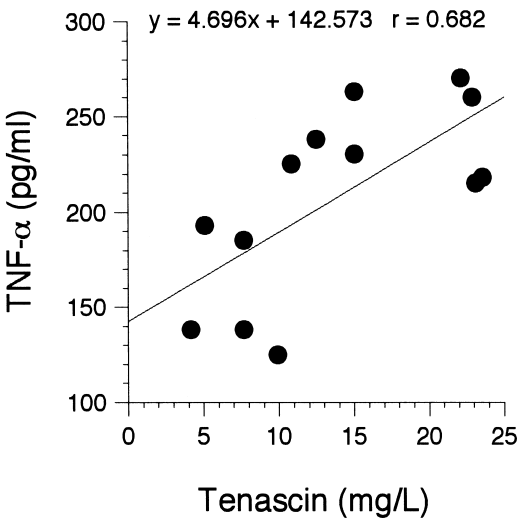

Fig. 3 Correlation between plasma level of tenascin and TNF-α level.

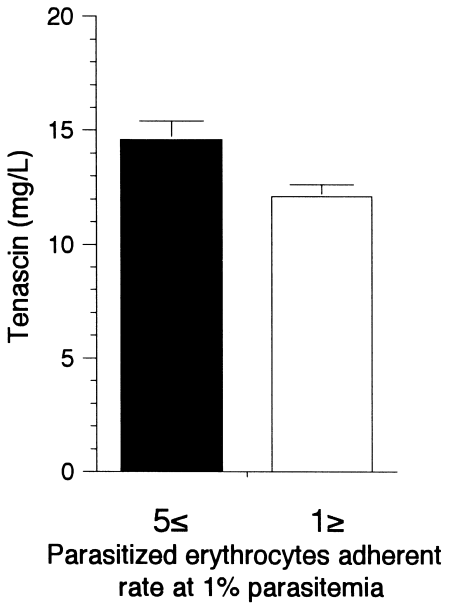

Fig. 4 Correlation between plasma tenascin level and parasitized erythrocytes binding ratio to the C32 melanoma cells