ELECTRON MICROSCOPY OF THE HUMAN BRAIN IN CEREBRAL MALARIA

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INTRODUCTION

One characteristic of the erythrocytes infected by asexual *Plasmodium falciparum*, that caused the most morbidity and mortality in man, is the disappearance of the infected erythrocytes from peripheral blood during maturation from late trophozoite to mature schizont. Previously only young parasites have been examined by electron microscope and described in detail (Ladda *et al.*, 1966; Trager *et al.*, 1966; Smith *et al.*, 1969; Miller, 1972). Several electron microscopic observations have shown that older parasites are sequestered in capillaries of heart, adipose tissue, skeletal muscle, submucosa of small intestine and other organs (Clark *et al.*, 1949; Desowitz *et al.*, 1969; Miller *et al.*, 1971; Gutierrez *et al.*, 1979) and may lead to vascular obstructions.

Sequestration of the infected erythrocytes within cerebral vessels has also been thought to play some part in the obstruction of these vessels and produce the symptoms of cerebral malaria.

Cerebral malaria is the most important form of severe falciparum malaria. In Thailand, it occurs in about 80% of severe cases of falciparum malaria admitted to the hospitals and the mortality rate is still high. The most striking features of cerebral malaria in man are the development of severe and diffuse disturbance of cerebral function (Harinasuta *et al.*, 1982). The usual victims are children who lived in areas where malaria is endemic and adult visitors to those areas (Rest, 1983).

Various pathogenic mechanisms have been suggested to be the cause of cerebral malaria. Maegraith and Fletcher (1972) suggested cerebral vascular stasis and oedema secondary to leakage from cerebral capillaries. Other investigators suggested sequestration of parasitized erythrocytes in capillaries (Yoeli *et al.*, 1974; Warrell *et al.*, 1982; Rest, 1983), margination and attachment to endothelial surfaces of parasitized erythrocytes (Trager *et al.*, 1966; Miller *et al.*, 1971; Miller, 1972; Gutierrez *et al.*, 1976; Udeinya *et al.*, 1981), decreased deformability of infected red cells causing obstruction of capillaries (Miller *et al.*, 1972), a prominent role for DIC (Jaroonvesama, 1972) and immuno-electron microscopy of cerebral malaria in golden hamsters suggested that thymus-dependent humoral response is involved in the pathogenesis of cerebral malaria (Rest and Wright, 1979).

Speculations on the mechanism of cerebral malaria which have been based on electron microscopic observations, have been only from experimental animal studies (Miller *et al.*, 1971; 1972; Gutierrez *et al.*, 1976; Rest and Wright, 1979; Rest, 1983). Interesting that, by light microscopic observation, many parasitized red blood cells packed in cerebral capillaries in patients who died of cerebral malaria has been reported (Spitz, 1946).

This study describes the ultrastructural aspects of *Plasmodium falciparum* and the parasitized red cells interaction with the endothelial cells in the human brain, obtained 3 hours post mortem from a patient who died

of cerebral malaria, pulmonary oedema and pneumonia.

MATERIALS AND METHODS

Case history

A 13-year-old Thai boy was admitted to the Bangkok Hospital for Tropical Diseases, for fever of 6 days, deep jaundice and coma. One day before admission he was treated for malaria at a private clinic but there was no improvement. On admission he had high fever, deep jaundice and was dehydrated and unresponsive. Blood examination revealed asexual *Plasmodium falciparum* of 7.3% and low platelets. Quinine HCl was given intravenously. On the second day the parasitaemia slightly increased, then gradually decreased on the fourth day, the parasitaemia was 0.9%, and the platelets were still low. His condition slightly improved but on the following day there was renal insufficiency, and pulmonary oedema with pneumonia. The patient died on the fifth day of hospitalization.

Autopsy was carried out 3 hours after death, small pieces of cerebrum from the area of white matter and small pieces of choroid plexus from lateral ventricle were obtained. They were rapidly minced and fixed in modified Millonig's phosphate buffered formalin (Carson *et al.*, 1973) at room temperature for 2 h. After fixation the tissues were washed 3 × 15 min with 0.2M Millonig's phosphate buffer containing 4% sucrose, post-fixed for another 2h in 1.5% OsO_4. Dehydration was carried out in acetone, tissues stained with 2% uranyl acetate in 70% acetone was included before infiltration with propylene oxide and embedding in Epon 812. Sections were cut with glass knives on LKB ultramicrotome and stained with lead citrate (Reynold, 1963) before examination with a Hitachi HU-12A electron microscope.

RESULTS

Gross Finding: The brain weighed 1400 gm. The external appearances of the brain showed marked oedema and congestion. With cross section, there were peticheal haemorrhages in white matter of the cerebrum. The cerebellum was also congested and oedematous.

Electron microscopy

Brain: Extensive vacuolization of brain substances was noted. Cerebral vessels and myelinated nerve fibers were seen. There was no acute or chronic inflammatory cell infiltration. No fibrin or thrombus formation was observed.

The cerebral vessels were generally not remarkable, only the luminal surface membrane of the endothelial cells showed advance degeneration, the cytoplasmic organelles looked well preserved and the basement membrane was in normal limit.

The lumen of these vessels were packed with parasitized red cells and non-parasitized red cells (Fig 1).

The endothelial cells showed moderate swelling and ballooning bulging into the lumen. In one section of the choroid plexus, a striking feature was the attachment of platelets to the active endothelial cell (Fig. 2).

Occasionally, endothelial vesicular membranes were seen. These protruded into the lumen and some were seen free in the lumen (Fig. 3a, 3b).

Erythrocyte : Uninfected erythrocytes had smooth surface but irregular in shape. Only a few extravascular erythrocytes were seen. The cytoplasm was more electron-dense compared to those infected erythrocytes.

Parasitized erythrocyte : Erythrocytes infected with *Plasmodium falciparum* were deformed, with distorted outline. The surface

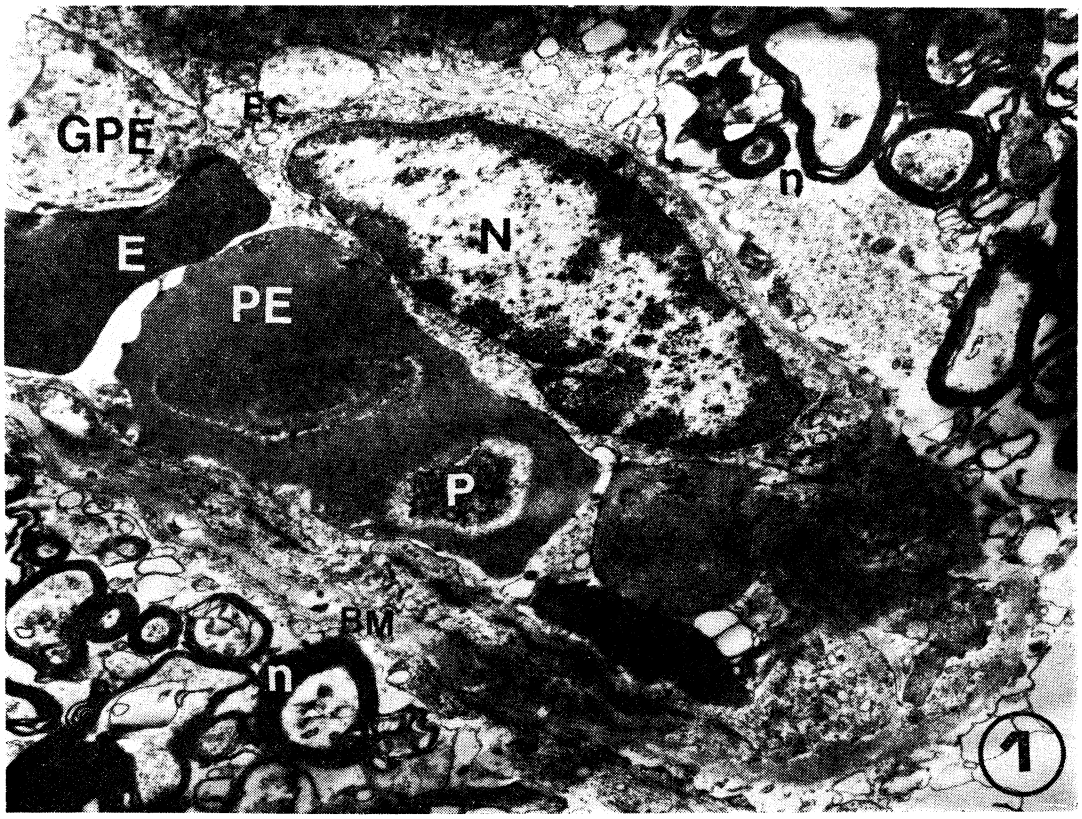

Fig. 1—Electron micrograph of a cerebral vessel showing several erythrocytes, some of which contain parasites (PE). The capillary shows moderate increase in size of endothelial cell (EC) and swollen nucleus (N). × 11,250

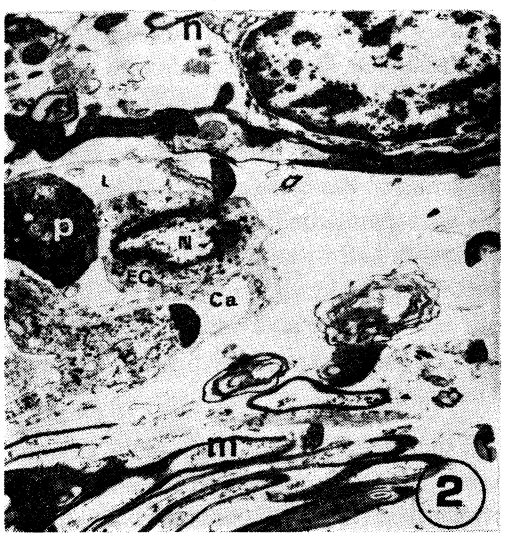

Fig. 2—Choroid plexus. A swollen endothelial cell (EC) is in close contact with platelet (p). Nucleus (N) of endothelial cell is bulging into the lumen. × 6,800

Abbreviation

BM	basement membrane
E	erythrocyte
EVM	endothelial vesicular membrane
K	knob
MC	Maurer's cleft
N	nucleus
P	parasite
p	platelet
Ca	capillary
EC	endothelial cell
GPE	ghost parasitized erythrocyte
L	lumen
m	microvilli
n	nerve
PE	parasitized erythrocyte
v	vesicle

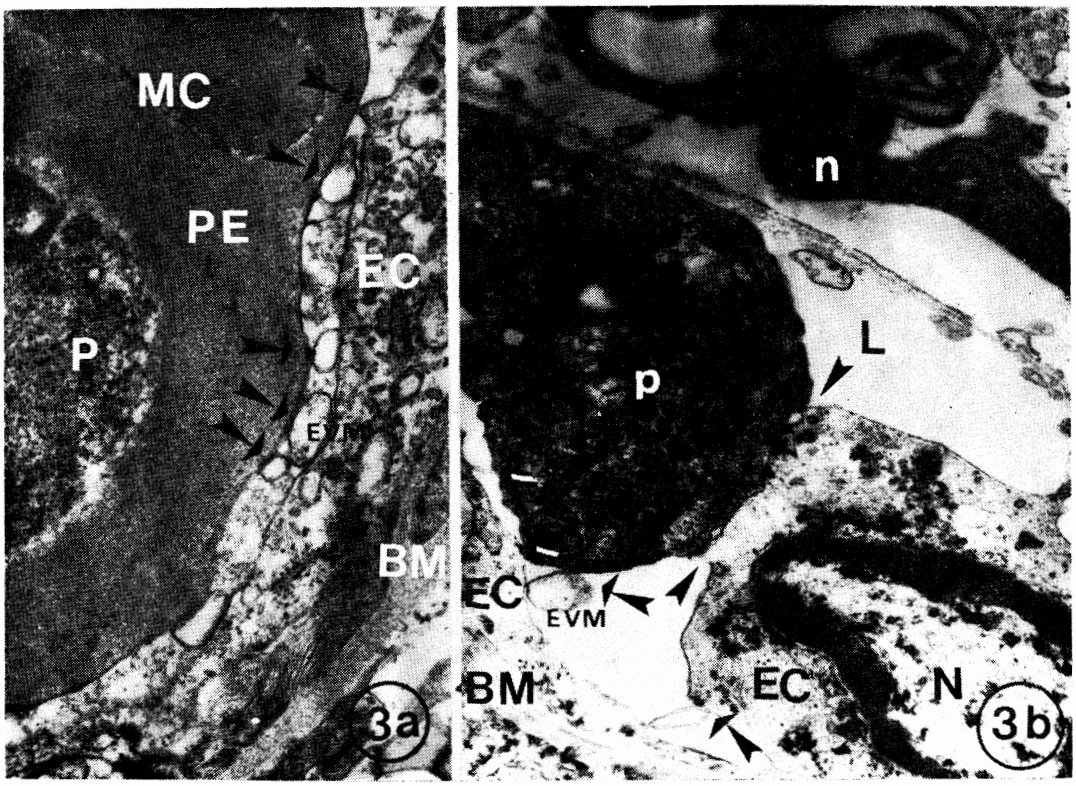

Fig. 3a, b—Endothelial vesicular membrane (EVM). Note the close contact of EVM with parasitized erythrocyte (3a) and with platelet (3b) (arrows). 3a × 22,500 ; 3b × 18,000

membrane of the parasitized cells were dotted with tiny electron-dense excrescences, as called "knob" by many investigators. The cytoplasm was less electron-dense than that of the normal erythrocyte. Maurer's clefts were presented in the cytoplasm, no connection of Maurer's cleft and knob was observed (Fig. 4, 5).

In some instance, "ghost" parasitized cells were seen with distorted outline and the presence of many knobs on their surface (Fig. 5).

Parasite : The parasites seen were mostly characterized by abundant content of ribosomes and poorly defined nuclei. The trophozoite was bounded by parasitophorous vacuole. Pigment vesicles and cytostomes were also seen in the cytoplasm of the trophozoites, mature schizonts were seen occasionally. In many instances the parasites showed morphological signs of degeneration (Fig. 4).

Endothelial and parasitized erythrocyte interaction : The close contact of endothelial cells with parasitized erythrocytes were seen at site with and without knob. At the site of contact without knob, there is no gap between both cells and also no evidence of membrane fusion, it seems likely that this was the result of tangential cut.

At the site of contact with knob, adjacent to this point of contact, there were large gaps between the distorted parasitized cell membrane and the endothelial cell membrane.

There was no contact between endothelial

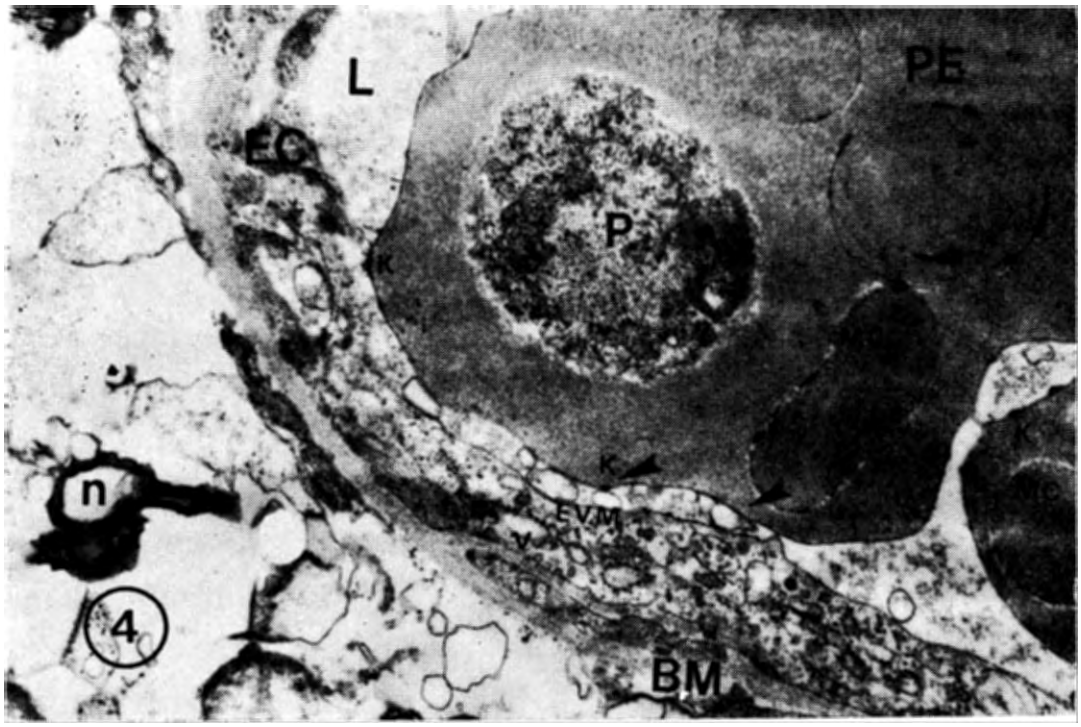

Fig. 4—A cerebral vessel. A parasitized erythrocyte (PE) within the lumen (L) and the surface knobs (K) appear to be attached to the endothelial membrane (arrows). Maurer's clefts (MC) are present in the erythrocyte cytoplasm. The parasites (P) appear to degenerate. × 18,500.

cell membrane and parasitized ghost cell at the site with or without knob (Fig. 5).

In many specimens the luminal surface membrane of endothelium showed advance degeneration. It is of interest that there were vesicular membranes attached to the parasitized erythrocyte and they seemed to join the parasitized cell and the endothelial cell to stick together at the site with and without knob. The appearance of these membranes which resemble vesicular membranes seem to occur from the endothelial cell, some of them were seen free in the vascular lumen (Fig. 3a, 4).

In the choroid plexus, similar vesicular membranes were also seen and they were in intimate contact with the platelets and endothelial plasma membrane (Fig. 3b).

DISCUSSION

In our studies there is no evidence of inflammation, peticheal haemorrhage or extravascular pathology. Even though gross findings showed oedema of the brain, the degeneration around cerebral vessels could not be related to significant perivascular pathology.

There was no fibrin or thrombus formation as described by Jaroonvesama (1972). So intravascular coagulation is not the cause of cerebral pathology in this case.

Our study on ultrastructural observation of *P. falciparum* in human brain did not show any major significance difference from the ultrastructure of *P. falciparum* in human erythrocytes described previously (Ladda

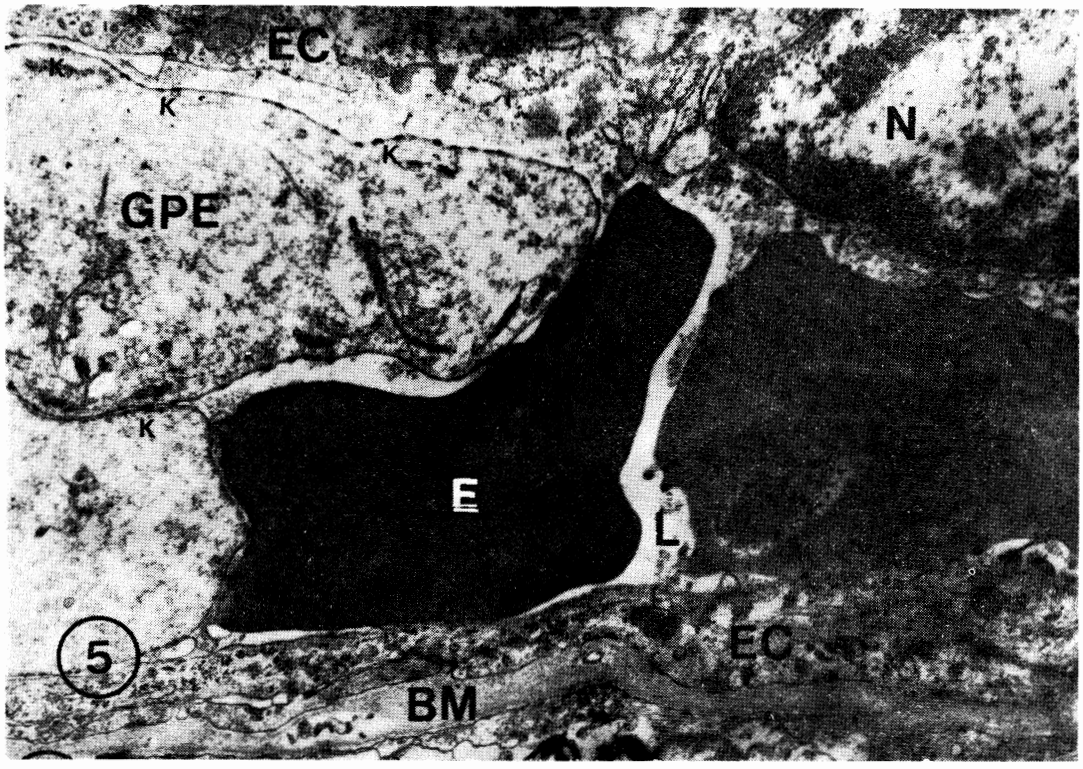

Fig. 5—The "ghost" parasitized erythrocyte (GPE) within a cerebral vessel is recognizable because of the numerous surface knobs (K). The parasitized erythrocyte adheres to the endothelium via knobs, but no adherence of the ghost parasitized erythrocyte and the endothelium. × 16,700.

et al., 1966; Trager *et al.*, 1966; Luse and Miller, 1971; Miller, 1972). The parasite in the erythrocyte in cerebral vessels showed morphological signs of degeneration which may be due to quinine given to the patient for 4 days or just simple post mortem degeneration. The drug had apparent effect on the reduction of parasitaemia. Thus, it is possible that these parasites were injured somewhere in the vascular circulation before sequestration in the cerebral vessels. This needs more investigation.

One interesting clinical feature is the low platelet. The sequestration of the platelet in the vascular endothelium may result in decrease in platelets in the peripheral blood. In choroid plexus vessel, platelet sticking to marked swollen endothelial cell was seen (Fig. 2, 3b).

Several pathogenesis of falciparum malaria have been postulated by several investigators. The most frequently description is the presence of parasitized erythrocytes in small blood vessels in the brain with apparent blockage of these vessels (reviewed by Aikawa *et al.*, 1980). The intimate contact between parasitized erythrocyte membranes and endothelial cell membranes seen in our study suggests the cause of tight packing of the parasitized erythrocytes in the cerebral vessels. The presences of surface excrescences as called "knob" on host erythrocytes that caused adhesion to vascular endothelium was first proposed by Rudzinska and Trager (1968), and later by many investigators (Miller *et al.*, 1971; Gutierrez *et al.*, 1976; Udeinya *et al.*, 1981). In our study, we confirm their findings.

The erythrocytes infected with immature trophozoites of *P. falciparum* show very few knobs on their surface membrane. The number of knobs increases as the parasite matures. There is no knob on the surface membrane of erythrocyte infected with gametocyte (Aikawa, 1983). The role of the knob in binding to endothelial cells during sequestration has been considered and reported that there are other factors besides the morphological alteration of parasitized erythrocytes. One factor is the erythrocyte membrane covering the knobs is immunologically different from the rest of the erythrocyte membrane (Kilejan *et al.*, 1977), more likely that the knobs contain materials of parasite origin. It has already been shown that prolonged *in vitro* culture of *P. falciparum* led to the loss of knobs from the surface of the infected cells (Langreth *et al.*, 1979).

However, not all knobby parasitized cells will bind, *Plasmodium malariae*-infected erythrocytes also develop knob-like protrusion, yet they remain in peripheral blood circulation (Smith and Theakston, 1970).

Barnwell *et al.*, (1983) have reported that parasites which bound via knobs to endothelial cells *in vitro*, after one or two passaged in splenectomized monkey, parasites were recovered which still expressed knobs on the surface of the infected cells but failed to bind to endothelial cell *in vivo* and did not sequester *in vivo*.

The finding in this study revealed that the ghost parasitized erythrocyte also presented many knobs on its surface but no evidence of contaction. Therefore, we confirm the hypothesis that, there are factors responsible for the morphological alteration, and others responsible for the capacity of the parasitized cells to bind to endothelium.

Besides of the attachment via knob, particular interest is the finding of endothelial vesicular membranes that seemed to be responsible for the attachment of the parasitized erythrocytes to the endothelium. These endothelial vesicular membranes also showed the same attaching action to the platelet in small vessel of choroid plexus, thus that might be the cause of platelet sequestration and obstruction of cerebro-spinal fluid.

The endothelial vesicular membranes as seen in our study were similar to the extensions of the endothelial cells as seen in the electron micrograph of Miller *et al.*, (1971). They reported that the mechanism for deep vascular schizogony in *P. knowlesi* differed from that in *P. falciparum*, only cerebral vascular involvement appeared similar to that in fatal cases of *P. falciparum* infection. No knob-like protrusions were seen on the surface of erythrocyte infected with *P. knowlesi*, the membrane of the parasitized red cell conforms directly to the endothelium and some of them attach to the extension of endothelial cell.

The endothelial vesicular membrane or the so called "extension of the endothelial cell" or "pseudoprotrusion of the endothelial cell" by the other authors can be regarded as the first sign of endothelial cell transformation into active macrophages. The significance of such changes is not well understood.

In conclusion, from our study there is morphological evidence of adhesion of parasitized erythrocytes to the endothelium of cerebral vessels via their surface knobs, endothelial vesicular membranes involved in the mechanism of adhesion, leading to vascular obstruction and disturbances of microcirculation in the brain, resulting in hypoxia. The evidence of platelets attached to the injured vascular endothelial cell may play a role in obstruction of cerebro-spinal fluid, leading to cerebral oedema, and also responsible to low circulating platlets.

SUMMARY

Ultrastructure of erythrocytes infected with *Plasmodium falciparum* in human brain, obtained 3 hours post mortem revealed gross distortion of host red cells with abnormality of the red cell surface. The superficial alterations of the parasitized cells as knob-like protrusion appear to be the sites of attachment to vascular endothelium. There was evidence of platelets sticking to the injured endothelium. The endothelial vesicular membrane is in close adhesion to the parasitized red cell, and also to the platelets involved in this mechanism. Thus, explaining the sequestration of parasitized red cell and obstruction in cerebral microcirculation, cerebral oedema and low peripheral platelet count.

The was no evidence of inflammation, fibrin or thrombus formation observed in our studies.

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