P B Fernando Memorial Oration

Cerebellar involvement in falciparum malaria: investigation of an epidemic

H J de Silva[1]

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Madam President, family of Prof. P B Fernando, Fellows and Members of the College, my teachers, ladies and gentlemen. It is an honour to be awarded this oration dedicated to the memory of one of the greatest medical academics this country has produced, in this, the Silver Jubilee of the College. Prof. Fernando, an old boy of St Benedicts college, Colombo, qualified as a Licentiate in Medicine and Surgery in 1924, and had his postgraduate training at University College hospital, London. In 1936, he was appointed the first Professor of Medicine at the Ceylon Medical College which later became the Faculty of Medicine, of the University of Ceylon. After many years of distinguished service, he died in 1965. Although I did not know him personally, I have been fortunate enough to know some of those who were taught by him and worked in his Department. From their stories about him and by reading some of his many publications, there is no doubt that here was a brilliant clinician, keen researcher, dedicated and beloved teacher and a kind hearted gentleman. His research interests covered a wide field and included malaria, and had he been amongst us, my talk tonight would probably have interested him.

Introduction

Falciparum malaria can give rise to a multitude of neurological manifestations. These may include psychoses, confusion, convulsions, coma, meningism, involuntary movements[1,2,3,4], disorders of conjugate gaze[5], cranial nerve palsies, extrapyramidal tremor, polyneuropathy, mononeuritis multiplex, Guillain-Barre syndrome [3,4,6,7,8], and myopathy[9,10]. Many of these manifestations including psychoses, extrapyramidal symptoms and flaccid paraparesis suggestive of Guillain-Barre syndrome were in fact described by Prof. P B Fernando and a colleague, Dr. Sandarasagara, in 1935[11]. Although cerebral malaria is by far the most important and potentially life threatening neurological complication, this paper focuses attention on a lesser known target organ in falciparum malaria — the cerebellum.

1 *Senior Lecturer and Head, Dept. of Medicine, Faculty of Medicine, University of Kelaniya, Talagolla Road, Ragama.*

Cerebellar Dysfunction During Attacks of Falciparum Malaria

The first descriptions of cerebellar signs in falciparum malaria in the English language medical literature date back to the beginning of this century. Deaderick[12] reported cerebellar ataxia of short duration in patients with cerebral malaria who were recovering from coma. Later, there were many isolated reports of cerebellar signs occurring during attacks of falciparum malaria[13], including a patient from Sri Lanka[11].

In falciparum malaria, cerebellar dysfunction has been reported to occur either during cerebral malaria as part of a 'global encephalopathy' [13,14,15] or as a selective impairment of cerebellar function in patients who have no other features of cerebral involvemant[14,16,17]. Illangasekera and de Sylva in 1976[14] described two cases of falciparum malaria with cerebellar signs. One had left sided cerebellar signs without any other neurological deficit during what seemed to be a mild attack of malaria, and the other had bilateral cerebellar involvement together with impaired consciousness and pyramidal signs. Both patients recovered after treatment with intravenous chloroquine, with complete disappearance of the neurological signs. Recently, Chaine *et al* [15], reported a patient with falciparum malaria who had isolated cerebellar signs, but whose electroencephalogram suggested a more diffuse encephalopathy. There was rapid improvement of cerebellar ataxia following treatment with mefloquine. Several isolated cases of cerebellar ataxia complicating falciparum malaria, in children [17,18,19] and an adult[16], have been documented in India. Cerebellar dysfunction, which was the only neurological abnormality, was accompanied by fever. The patients responded dramatically to treatment with oral chloroquine, with complete neurological recovery occurring within 48 hours of starting treatment.

Further evidence that the cerebellum can be involved during attacks of falciparum malaria comes from autopsy studies. In addition to clogging of cerebellar cortical capillaries with parasitised red blood cells, lesions such as infarcts, perivascular haemorrhages, shrinkage of Purkinje cells and perivascular clusters of microglia have been described in the cerebelli of patients dying of cerebral malaria[20,21].

Thus, although uncommon, there is clear clinical and pathological evidence that falciparum malaria can give rise to cerebellar dysfunction. Furthermore, cerebellar dysfunction does not seem to be related to the severity of malaria and can occur independently of cerebral involvement.

Delayed Onset Cerebellar Ataxia (DCA) Complicating Falciparum Malaria

So far, what had been described was cerebellar dysfunction occurring during attacks of falciparum malaria. Then, in 1986, de Silva *et al* [22] reported 5 patients with a self-limiting, midline, cerebellar syndrome. The factor common to all of them was a well documented attack of *Plasmodium falciparum* malaria, which had been 'successfully' treated with chloroquine. There was a delay of 1 to 4 weeks between the attack of malaria and the onset of cerebellar signs, and ataxia developed after an afebrile period. All of them had predominantly midline cerebellar signs, with gait and truncal ataxia being the most prominent findings. There was no evidence of any cerebral involvement, and there was no fever or parasitaemia during the cerebellar, dysfunction. Their recovery was gradual and spontaneous and they were all asymptomatic within 8 weeks of the onset of ataxia. Routine haematological and biochemical investigations. CSF examination including culture, electroencephalography and skull X-rays were normal. There was no serological evidence to suggest Japanese Encephalitis Virus infection. It was suggested that cerebellar dysfunction was likely to be a delayed complication of falciparum malaria.

Although this was the first published report, Senanayake *et al* [23] had reported 2 patients presenting with cerebellar symptoms of two months duration in whom gait ataxia had developed on about the fourth day of a febrile illness which had been diagnosed as malaria and treated with chloroquine. The patients were afebrile during the cerebellar dysfunction. Their blood films showed gametocytes, but not asexual stages, of *P. falciparum*, which was taken as proof of recent falciparum malaria. The symptoms had gradually improved and they had become asymptomatic within 4 months of the onset of ataxia. Although the timing of onset of cerebellar symptoms in relation to the onset of fever in the attack of malaria was shorter than in the patients that de Silva *et al* reported, the rest of the clinical features were similar. Variations in timings such as this are only to be expected, especially when describing a new complication. It is therefore very likely that the same complication was being described by both authors.

Following this, Senanayake[24] reported a further 12 patients, and de Silva *et al* [25] reported 8 more patients with DCA following an attack of falciparum malaria. There have been many other reports of a similar syndrome[10,26,27,28,29], all except two case reports[28,29], originating from Sri Lanka. Although the great majority of patients who had this syndrome were from the North Central province, there were some who lived in or visited other parts of the country which are hyperendemic for malaria. Of the two case reports of DCA that did not originate from Sri Lanka, one had pancytopenia in addition to cerebellar ataxia[29], and the other was poorly investigated[28].

These reports, especially when taken together, strongly suggested a causal relationship between falciparum malaria and the subsequent development of cerebellar ataxia. However, several other aetiological possibilities existed, at least theoretically, that needed to be excluded before reasonable assumptions about cause and effect could be made. None of the cases reported had been investigated in any great depth, and especially not with a view to excluding other causes of self-limiting cerebellar dysfunction. A more detailed investigation of this interesting phenomenon was necessary.

Clinical features of the syndrome

Between December 1986 and November 1987, 32 patients who were admitted to the General Hospital, Colombo, with DCA complicating a documented attack of falciparum malaria were prospectively studied. A standard protocol was prepared, and clinical data and results of investigations were entered into a data sheet.

There were 29 males and 3 females, aged between 16 to 52 years (median 27). Of the 32 patients, 19 were permanent residents, and 7 were regular visitors to areas highly endemic for malaria (Figure 1). The other 6 patients had probably contracted malaria during a single visit to an endemic area. Seventeen of the 19 permanent residents and 4 of the regular visitors had suffered attacks of malaria in the past (range 1 to about 30), from which they had all recovered uneventfully after being treated with antimalarials. Cerebellar dysfunction was related only to the last attack. This was a well documented attack of uncomplicated falciparum malaria, which was 'successfully' treated with chloroquine, and all the patients had been afebrile for between 5 to 22 days (median 8) before the appearance of cerebellar dysfunction. The onset of ataxia was sudden, occurring a median 14 days (range 7 to 28) from the onset of fever and the disability was maximal in 2 to 14 days (median 5). On admission to the General Hospital Colombo, they had been ataxic for between 2 to 21 days. The cerebellar signs were predominantly midline, truncal and gait ataxia being pronounced in all 32 patients (27 could not even sit up, and 5 could sit up but not stand up or walk). Other findings were dysarthria in 16, an abnormal finger-nose test in 14, nystagmus in 8, dysdiadochokinesia in 2 and pendular

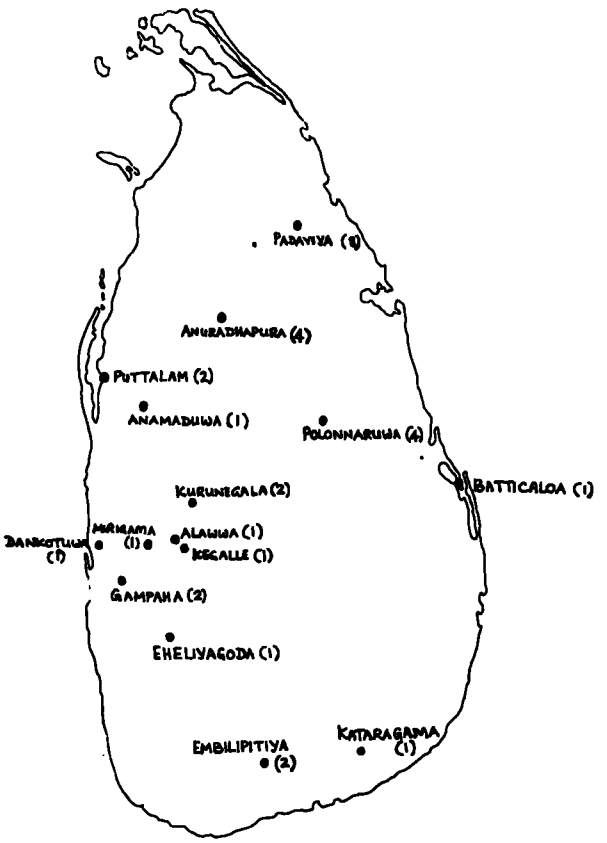

Figure 1. **Locations where malaria was probably contracted by patients with DCA** (number of patients indicated within parentheses)

knee jerks in 1. None of the patients were febrile on admission or during the course of the cerebellar illness. No other abnormalities were found on clinical examination except for a palpable spleen in 7 of the patients. They were conscious, rational and alert, and had no signs indicating cerebral or pyramidal tract involvement. There was no clinical evidence of neoplasia. In all patients, there were no features suggestive of an alternative cause of cerebellar pathology. Particular attention was paid to the history of alcohol abuse, exposure to heavy metals, drug history, recent vaccination, other febrile illnesses (including exanthemata), and the family history: all of which were negative (except the use of chloroquine to treat the attack of malaria). The recovery was spontaneous and complete in 15 to 56 days (median 28). Twenty of them continued to be asymptomatic at follow up 3 months after recovery. The other 12 were lost to follow up.

Investigations

During ataxia, the blood films of each patient were negative for asexual stages of *P. falciparum* on at least 3 occasions. This was not surprising as they had all been treated with chloroquine and were afebrile. However, 6 patients had gametocytes of *P. falciparum* in their blood films. The following investigations were within normal limits: full blood count, blood glucose, urea, creatinine and electrolyte concentrations, liver function tests, thyroid stimulating hormone (TSH) levels, Widal's test, chest X-ray and electroencephalography. Brain CT scan with contrast enhancement which was performed on 8 patients was also normal. Cerebrospinal fluid (CSF) protein concentration was median 30mg/100ml (range 15 - 40), and CSF cell counts were within normal limits. CSF culture for bacterial pathogens was negative, and there was no evidence (IgM or IgG Abs) of Japanese Encephalitis, Coxsackie B (types 1-6) or Echo virus infection either in the CSF or serum. The erythrocyte sedimentation rate was variable [median 16mm (range 5-50)].

Plasma chloroquine levels were assayed by a colorimetric method in 20 specimens (12 patients and 8 controls). The 12 patients (10 males and 2 females), were aged 16 to 52 years (median 29) with cerebellar ataxia 18 to 26 days following a documented attack of falciparum malaria. Blood was collected on admission 22 to 31 (median 26) days after onset of the malarial attack. The controls were 7 males and 1 female, aged 23 to 46 years (median 25), who 21 to 26 (median 24) days after the onset of an attack of falciparum malaria had not developed cerebellar symptoms. The chloroquine levels were less than 60µg/l in all specimens tested, with no significant difference in levels between patients and controls.

The reasons for reporting this series of 32 patients are two-fold. Firstly, to describe in detail the clinical picture of DCA complicating falciparum malaria. These results confirm the clinical features of the syndrome described in previous reports[22,24,25], which is of a self-limiting, acute onset, predominantly midline cerebellar lesion, without any evidence of cerebral involvement, which occurs 7 to 28 following the onset of an attack of otherwise uncomplicated falciparum malaria, and which lasts at most about 8 weeks. Secondly, this is the first study that attempts to exclude, as far as possible, other likely causes of a self limiting cerebellar ataxia by an 'in-depth' investigation.

As all patients with this syndrome were previously treated with antimalarials, the possibility that DCA may have been due to a toxic effect of chloroquine has been suggested[24,27]. In this study, plasma chloroquine levels were lower than 60µg/l in all the samples tested, with no differences between patients and controls. Adverse reactions to chloroquine usually develop only when plasma concentrations exceed 250µg/l[30,31]. Furthermore, several of the patients described by Senanayake[24] had in fact been treated with amodiaquine and had not recei-

ved any chloroquine, and DCA did not occur in any patient following vivax malaria which was treated with chloroquine. On the same grounds a toxic contaminant in the drug can be discounted. Although there are descriptions of acute cerebellar ataxia following a wide variety of infections[32,33,34], there was no evidence of an infection other than the episode of malaria in the patients in this series. Therefore, as other likely causes of a self-limiting cerebellar syndrome have been excluded as far as possible, it is reasonable to assume that a causal relationship exists between the attack of falciparum malaria and DCA. It must be re-emphasized that this complication has not been described in patients with vivax malaria, which is the commoner type of malaria in Sri Lanka[35]. Therefore, it seems that the DCA is specifically related to falciparum malaria.

The selective involvement of the cerebellum and especially the delay between the onset of fever and the onset of cerebellar dysfunction suggest that immune mechanisms are likely to be involved in the pathogenesis of this syndrome. Senanayake[24] further suggested that a new strain of *P. falciparum* may be responsible. Though most of the patients in the present series came from the North Central part of Sri Lanka, there were others from many different localities. However, cross transmission of a new strain of *P. falciparum* among different localities was possible. In this study, as in previous reports[22,24,25], the majority of patients affected were young males. This probably only reflects the general pattern of malaria infection in Sri Lanka[35]. Nevertheless, because not all persons who contract falciparum malaria develop DCA, host susceptibility factors, which are yet to be defined, may also be important in the pathogenesis of the syndrome.

Pathogenesis

It has been frequently suggested that immune mechanisms are likely to be involved in the pathogenesis of DCA complicating falciparum malaria[4,24,25]. The delay in the onset of cerebellar dysfunction, the selective involvement of the cerebellum, and the favourable response observed in patients treated with systemic steroids — which led to shortening of the duration of ataxia[24], all favour this possibility. However, apart from these clinical observations, no other evidence has been put forward to support the hypothesis.

To test the hypothesis that immune mechanisms are responsible for DCA complicating falciparum malaria, evidence for immune activation in patients with this syndrome was sought by assessment of cytokine concentrations in the serum and cerebrospinal fluid (CSF)[36]. Cytokines are mediator substances which are mainly released by activated cells of the immune system. Increased concentrations, of these substances would reflect immune activation. Next, the possible involvement of humoral immune mechanisms was investigated, by testing for the presence of immune complexes and antibodies directed against the cerebellum in these patients, using immunofluorescent and immunohistochemical techniques[37].

Assessment of Cytokine Concentrations

Patients

Twelve of the patients described above (10 males and 2 females) aged 16 to 52 years (median 29) with DCA were studied with their informed consent. Ataxia had developed 18 to 26 days after the onset of fever. In addition to other investigations performed as part of an initial evaluation, 5ml of venous blood was collected from each patient, on admission 22 to 31 (median 26) days after onset of the malarial attack, and after complete symptomatic recovery. 2ml of cerebrospinal fluid (CSF) was also obtained, during routine lumbar puncture for CSF culture and virology.

Control sera were obtained from 8 patients [7 males, 1 female; aged 23 to 46 years (median 25)], who 21 to 26 (median 24) days after the onset of an attack of falciparum malaria had not developed cerebellar symptoms. The attack was uncomplicated and successfully treated with chloroquine. The patients (controls) were asymptomatic and their blood films were negative for *P. falciparum* when the serum samples were obtained. They continued to be asymptomatic when seen 3 months later. Control CSF was obtained from 6 non-malarial patients [5 males, 1 female; aged 21 to 59 years (median 31.5)] who were being investigated for symptoms suggestive of nerve root compression. Their myelograms and routine CSF examination were both normal.

Cytokine assays

CSF samples were immediately centrifuged, and the supernatants, together with serum samples from patients and controls were stored at -20°C in Sri Lanka, transported in dry ice to Oxford, and stored at -70°C until cytokine assay. Tumour necrosis factor α (TNFα) and interleukin 6 (IL6) were measured by an enzyme amplified sensitivity immunoassay (Medgenix Diagnostics, Belgium), and interleukin 2 (IL2) was measured by a sandwich enzyme immunoassay (T cell Sciences, Cambridge, Mass., USA). Briefly, the samples were pipetted into polystyrene microtitre wells (in 96 well plates) coated with the relevant mouse anti-human anti-cytokine antibody. Unreacted sample components were removed by washing with phosphate buffered saline. A mouse anti-human monoclonal antibody to the cytokine tested for was then added. After another wash an enzyme conjugated goal anti-mouse antibody was added, and the reaction was then

developed by adding o-phenylenediamine. Optical densities were measured by an automated dual beam ELISA reader at 450nm for TNFα and IL6, and 490nm for IL2, as recommended. A standard curve was plotted for each cytokine assayed using the standard solutions provided, and cytokine concentrations in the samples were then calculated. The assays were performed in duplicate for each sample of serum or CSF tested and the mean value was taken. The minimum detectable concentrations of these assays were: TNFα 3pg/ml; IL6 3pg/ml; and IL2 6pg/0.1ml. Results below these limits were assigned values of 2pg/ml or 2pg/0.1ml for statistical purposes. Grouped data were expressed as median and range. Differences between measurements were assessed by the Mann-Whitney U test. The significance of correlation was determined by the Spearman Rank Correlation test. A $p < 0.05$ was considered significant.

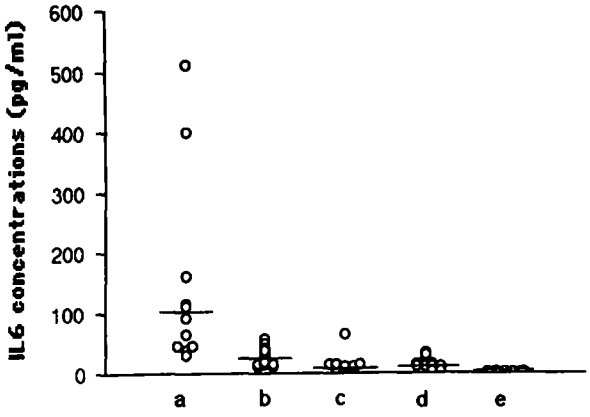

Figure 3. **IL6 levels in serum and CSF of patients and controls** (a = patient serum on admission, b = patient serum on recovery, c = control serum, d = patient CSF on admission, e = control CSF. Horizontal bars denote median)

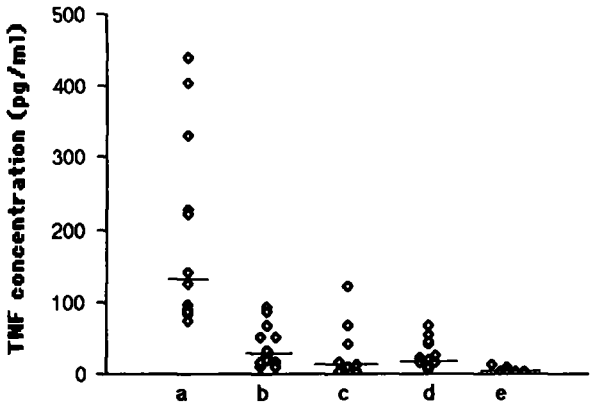

Figure 2. **TNFα levels in serum and CSF of patients and controls** (a = patient serum on admission, b = patient serum on recovery, c = control serum, d = patient CSF on admission, e = control CSF. Horizontal bars denote median)

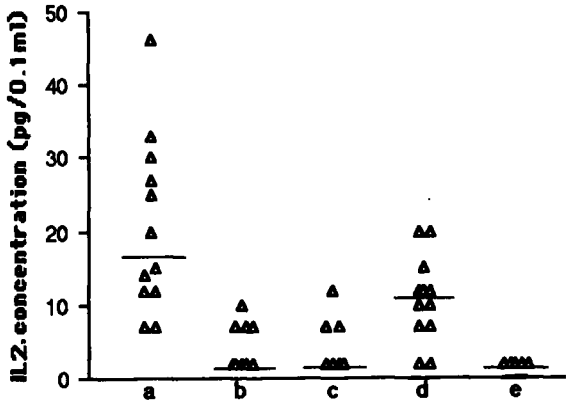

Figure 4. **IL2 levels in serum and CSF of patients and controls** (a = patient serum on admission, b = patient serum on recovery, c = control serum, d = patient CSF on admission, e = control CSF. Horizontal bars denote median)

Table 1. Cytokine levels in serum & CSF

	TNFα (pg/ml)	IL6 (pg/ml)	IL2 (pg/0.1ml)
Serum on admission (n=12)	132.5 (75-440)	101.6 (30-510)	17.5 (7-46)
Serum on recovery (n=12)	29 (10-93)	21.5 (10-58)	< 6 (< 6-10)
Control serum (n=8)	14 (< 3-123)	13.5 (5-65)	< 6 (< 6-12)
CSF on admission (n=2)	21.5 (7-67)	16.5 (10-35)	11 (< 6-20)
Control CSF (n=6)	< 3 (< 3-13)	4.5 (< 3-10)	< 6 (< 6)

Values expressed as Median (range)

Results

The results of the cytokine assays are shown in Table 1 and Figures 2,3 and 4. Although there was a wide distribution of observed cytokine concentrations (which may reflect the different times after onset of ataxia at which the samples were obtained), TNFα IL6 and IL2 concentrations were significantly higher in serum samples of patients with ataxia obtained on admission to hospital when compared with their concentrations in recovery sera and control sera ($p < 0.001$ for each comparison). This was despite the fact that sera from ataxic patients and controls were obtained after a similar time period from the onset of the attack of malaria. Cytokine concentrations in the CSF were also significantly higher in ataxic patients when compared with controls ($p < 0.001$ for each comparison). In patients with cerebellar dysfunction, serum concentrations of TNFα correlated with serum IL6 (rs=0.74, p=0.006) and IL2 (rs=0.59, p=0.04) concentrations, while CSF concentrations of TNFα correlated with CSF IL6 (rs=0.68, p=0.01) and IL2 (rs=0.57, p=0.05) concentrations. This suggests that there was a simultaneous increase in concentrations of the cytokines tested for.

This study, for the first time, provided evidence for immune activation during DCA complicating falciparum malaria. The self-limiting nature of the cerebellar dysfunction with full recovery within 8 weeks suggests a demyelinating process. However, due to the essentially benign nature of the condition, it has not been possible to perform pathological studies to confirm this. It is well known that malarial infections induce both cellular and humoral immune responses[4], and culture supernatants of stimulated T lymphocytes and TNFα have both been shown to have demyelinating activity in vitro[38,39]. TNFα is derived from macrophages/monocytes, IL2 derived from T lymphocytes, while IL6 can be produced by monocytes, T lymphocytes and endothelial cells[40,41]. In cerebral malaria, during active infection, high concentrations of TNFα are found in the patients serum, but not in the CSF[42,43]. The serum concentrations fall to normal levels within 4 weeks of recovery[43]. The observed simultaneous elevation of TNFα, IL2 and IL6 levels in both serum and CSF of patients during the cerebellar dysfunction, their subsequent fall as the symptoms improved, and the absence of raised cytokine levels in patients who did not develop cerebellar ataxia 21-26 days following an attack of falciparum malaria suggests that delayed cell-mediated immune activation (both macrophage/monocyte and T lymphocyte) played an important pathogenetic role in DCA. Furthermore, the increased cytokine levels in the CSF in patients in the present study, which is in contrast to findings in cerebral malaria where the CSF cytokine levels are normal despite high serum cytokine concentrations[43], may indicate local synthesis of these substances rather than passive transfer across the blood brain barrier.

Although cerebellar dysfunction appeared to be related to immune activation, the exact link between them cannot be deduced from the present data. Nevertheless, almost all reports describing this syndrome have originated from Sri Lanka[10,22,24,25,26,27]. Furthermore, several patients in those series, and 6 patients in the present study had previously had attacks of malaria without any complication. One possible explanation for this is that a new strain of *P. falciparum* was responsible for triggering the delayed onset immune response. The delayed onset of the cerebellar lesion and its very focal distribution within the brain raises the possibility of molecular mimicry between *Plasmodium* antigens and epitopes within the cerebellum.

The Search For An Antibody Directed Against The Cerebellum

It is possible that molecular mimicry existed between antigens of a certain strain(s) of *P. falciparum* and epitopes within the cerebellum. Antibodies formed during the attack of malaria might have then cross reacted with these cerebellar epitopes, giving rise to cerebellar dysfunction. Therefore, evidence was sought for the presence of antibodies directed against the cerebellum in patients with DCA complicating falciparum malaria.

Methods

During routine investigation which included lumbar puncture, 5ml of venous blood and 5ml of cerebrospinal fluid (CSF) were obtained from 19 patients with this syndrome (17 males, 2 females) aged 16 to 52 years (median 28) with their informed consent. Control sera were obtained from 6 patients who had not developed cerebellar symptoms 21 to 26 days after the onset of an attack of falciparum malaria. Control CSF was obtained from 6 patients who were being investigated for symptoms suggestive of nerve root compression during myelography (controls were the same patients mentioned in the cytokine study). CSF samples were centrifuged at 10,000 rpm for 30 minutes. The sediment which contained the glial cells was washed in phosphate buffered saline and resuspended in 500 ml. Thin smears were made on 12 well multitest microscope slides using this suspension (5-6 µl/well) and then air dried. Slides were stored at -70°C.

These glial cell preparations were examined for the presence of adsorbed malarial antigens and/or immune complexes using an indirect immunofluorescent technique. They were first incubated with immune sera known to have a high titre of anti-*P falciparum* antibody (1 in 2560) for 30 minutes, and then incubated with a 1 in 40 dilution of fluorescein conjugated goat anti-human immunoglobulin for 30 minutes at room temperature, and the slides were examined with a fluorescent microscope.

Evidence for the presence of anti-cerebellar antibodies in the serum and CSF was also investigated. For this, 5μm thick frozen sections of human cerebellum including sections of the cerebellar vermis, and cerebrum (negative control), were first incubated with undiluted sera or CSF supernatants of patients and controls for 60 minutes. The reaction was then developed using both 3 stage immunoperoxidase and ABComplex (avidin — biotinylated horseradish peroxidase complex) techniques. Briefly, after the primary incubation, the sections were washed with phosphate buffered saline to remove unreacted sample components. For the 3 stage peroxidase method they were then incubated, in turn, with peroxidase conjugated rabbit anti-human immunoglobulin and peroxidase conjugated swine anti-rabbit immunoglobulin for 30 minutes each at room temperature, with washes with phosphate buffered saline in between. The reaction was developed by incubating the sections for 5 minutes with diaminobenzidine. For the ABComplex method, after the first incubation with serum or CSF, the sections were incubated for 30 minutes each with biotinylated sheep anti-human immunoglobulin and ABComplex with washes in between. The reaction was then developed as before, with diaminobenzidine.

In all experiments, sections of cerebrum were used as tissue negative controls, and a non-related antibody (rabbit anti-mouse immunoglobulin) was used for the first incubation as a negative control for the primary antibody.

Results

All glial cell preparations were negative for adsorbed malarial antigens or immune complexes. There was no evidence of antibodies directed against the cerebellum in any of the serum or CSF samples tested immunohistochemically. This study did not provide evidence for antibody mediation in the pathogenesis of DCA complicating falciparum malaria. One possible explanation may be that the methods used lacked sensitivity. However, when considered together with the results of our study showing raised concentrations of TNF α, IL 2 and IL 6 in sera and CSF obtained from patients with this syndrome during cerebellar ataxia[36], it may be that cell mediated immune mechanisms are more important than humoral mechanisms in its pathogenesis.

Epidemiology of delayed onset cerebellar ataxia — evidence that a new strain of *Plasmodium falciparum* was responsible

After its first description in 1984[23], DCA complicating falciparum malaria appeared to reach epidemic proportions over the next few years in Sri Lanka. Two hospital based surveys on malaria done in 1986 showed that cerebellar ataxia was indeed a frequent complication of falciparum malaria. In an analysis of admissions for malaria at the Base Hospital Polonnaruwa in 1986, Wijesundere[10] reported cerebellar ataxia as the commonest neurological complication of falciparum malaria in adults. It did not develop in any patient with vivax malaria. Predominantly midline cerebellar signs developed a mean 5 days after the onset of fever, and complete neurological recovery occurred within 4 weeks of the onset of ataxia. In another study conducted in the first quarter of 1986, at the District Hospital Girandurukotte (in System C of the Mahaweli Development Programme), Edirisinghe *et al*[26] reported that about a third of patients who had falciparum malaria had evidence of cerebellar dysfunction. However, description of the clinical features are sketchy. Furthermore, no results of any investigation that may have been performed are mentioned in either of the two studies. This is an unfortunate shortcoming. The timing of cerebellar dysfunction in relation to onset of malaria fever was shorter when compared to earlier descriptions of the syndrome. Despite all this, it is more than likely that the same condition was being described by all the authors, albeit with slight variations in the clinical pattern which are, as mentioned previously, only to be expected when describing a new complication. This contention is further supported by the following facts: the clinical features were very similar in all patients; the patients were described during the same time period; most of the patients originated from one area (North Central region) of the country.

As mentioned previously, DCA complicating falciparum malaria appeared suddenly for the first time in Sri Lanka in 1984. Its incidence appeared to reach a peak and then decline; since 1990, hardly any cases have been reported. The outbreak of DCA appeared to parallel an epidemic of malaria which occurred in Sri Lanka between 1984 and 1988. The most significant feature of this epidemic was the disproportionate increase in *P. falciparum* malaria[35]. Therefore DCA may have been caused by a new strain of *P. falciparum*. To look for epidemiological evidence that would support this hypothesis, a retrospective hospital based survey was performed to investigate the incidence of this condition in the North Central region of Sri Lanka over a 6 year period[44].

Methods

The epidemiological survey was conducted in a region defined by 10 neighbouring Medical Officer of Health (MOH) divisions. These were Matale, Rattota, Naula, Dambulla, Galewala, Anuradhapura, Kekirawa, Kahatagasdigiliya, Padaviya and Tambuttegama (prior to 1991 this same area consisted of only 7 MOH divisions). These now make up the Matale and Anuradhapura Regional Director of Health Services (RDHS) areas (Fig.

5). This region was chosen by us, because the majority of patients with DCA complicating falciparum malaria had been reported from North Central Sri Lanka.

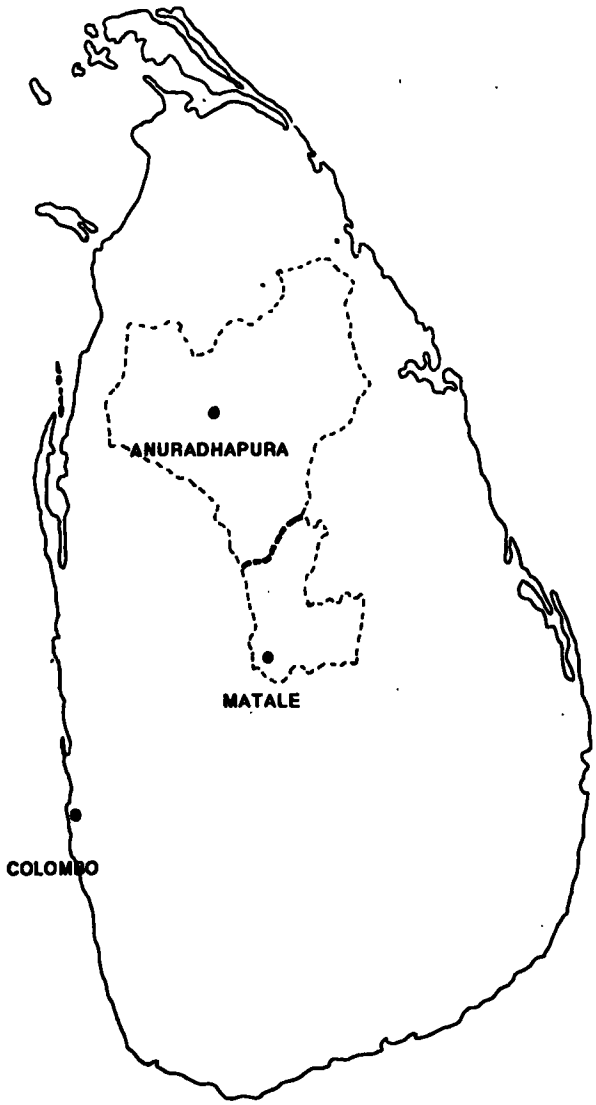

Figure 5. **Area in which the epidemiological study was conducted**

First, the pattern of malaria in the defined area between 1981 and 1991 was ascertained, by utilising the monthly returns of the Anti-Malaria Campaign from the 10 MOH areas. Thereafter, a selective sampling of the records of patients admitted to the Base Hospital Matale and General Hospital Anuradhapura from 1986 to 1991 was performed. The patient records of the previous years had been destroyed in both hospitals, and therefore no data were available for the period prior to 1986. The survey was limited to these 2 hospitals, which are the major hospitals serving the 2 RDHS areas under study, in order to avoid possible duplication of patient data. This was because, when contacted, the medical officers in charge of the smaller hospitals (District Hospitals and Peripheral Units) serving this area informed us that they always referred patients with DCA (and for that matter all patients with neurological problems) to a major hospital for assessment by a Consultant Physician. It was therefore assumed that data on most of the patients with this condition within the defined study area would be obtained by surveying only these two major hospitals.

Selective sampling consisted of examining the records of all patients admitted to both hospitals in the months of January, February, June and July from 1986 to 1991. Selective sampling was thought necessary because of the logistic problems anticipated in attempting to examine records of all hospital admissions for 6 years in two major hospitals. These 4 months were selected because the highest monthly incidence of malaria in this region occurs between November and January, with sometimes a smaller 'peak' in the middle of the year (monthly returns of the Anti Malaria Campaign from the MOH areas of Matale, Rattota, Naula, Dambulla, Anuradhapura, Kekirawa, and Kahatagasdigiliya, and for 1991, Galewala, Padaviya and Tambuttegama as well). It was felt that, under the circumstances, this method would probably give the highest yield of patients with DCA.

For purposes of this epidemiological survey, patients were considered to have had DCA complicating falciparum malaria if they fulfilled the following criteria (all 5 criteria had to be met for inclusion in the study):

1. Presence of selective and predominantly midline cerebellar dysfunction — prominent feature being ataxia, with no evidence of cerebral or pyramidal tract involvement.

2. Cerebellar dysfunction of a self-limiting nature — the patient at least partially recovered (could stand or walk) on discharge from hospital.

3. Absence of fever during cerebellar dysfunction.

4. Documentation of an attack of malaria between 7 to 30 days (one week to one month) prior to the onset of cerebellar signs — documentation as 'falciparum malaria' or 'malaria' where the type was not specified was accepted, because it is known that the cerebellar syndrome does not complicate vivax malaria[10,22,24,25].

5. No other possible cause for cerebellar dysfunction mentioned in the patient record.

Data obtained from the 2 hospitals were combined as it was found that there was no difference in the incidence pattern of the syndrome between the two hospitals during the period 1986 to 1991. The incidence was expressed per 1000 hospital admissions for the year concerned. This was to achieve a degree of uniformity in presenting the data, and is a valid method of expression because total admissions to the two hospitals did not vary greatly over the study period, and both the absolute numbers of patients with delayed cerebellar dysfunction as well as its incidence per 1000 admissions between 1986 and 1991 showed a similar pattern. The incidence pattern of DCA was then compared with the pattern of malaria in the area.

Results

Data obtained by utilising the monthly returns of the Anti-Malaria Campaign from the 10 MOH areas show that there was an epidemic of malaria in the two health regions surveyed between 1984 and 1988. The epidemic was largely due to an increase in the incidence of *P. falciparum* malaria, which reflected the national trend [35]. This is shown by both an increase in the absolute number of cases of falciparum and mixed infection malaria, and also an increase in the *P. falciparum* : *P. vivax* ratio in the area (Table 2, Figs. 6 and 7, mixed infections were grouped together with falciparum malaria). This ratio rose from 0.04 in 1984 to 0.32 in 1987, fell to 0.13 in 1988 and had risen again to 0.31 in 1991. Conversely, the *P. vivax: P. falciparum* ratio fell from 27.4 in 1984 to 3.6 in 1987, rose again to 7.3 in 1988 and then declined to 3.6 in 1991.

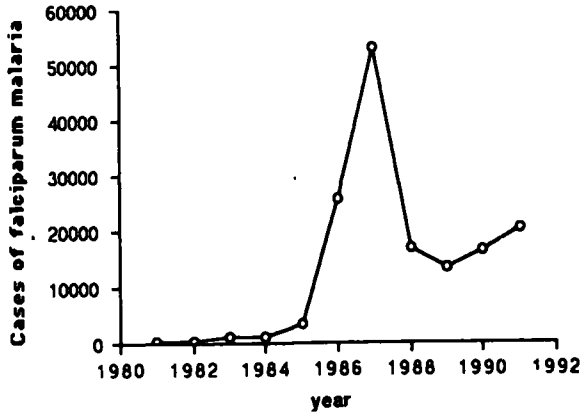

Figure 7. **Number of cases of falciparum malaria in the study area from 1981 — 1991**

In the hospital based survey, a total of 173,494 patient records were examined, and 215 cases of DCA were encountered. The absolute numbers of patients with DCA, total hospital admissions during the period of study, and the incidence of the syndrome between 1986 and 1991 are shown in Table 3. The incidence of DCA was 1.8 per 1000 admissions in 1986 and rose to a peak of 2.9 per 1000 in 1987 and declined to 1.9 per 1000 in 1989. This distribution corresponded closely with the epidemic of falciparum malaria in the area, which also reached a peak in 1987 (Fig. 8). However, despite a subsequent rise in falciparum malaria in 1991 (Figs. 6 and 7), the incidence of DCA continued to fall, and its incidence was near zero (0.05 per 1000 admissions) in 1991 (Fig. 8).

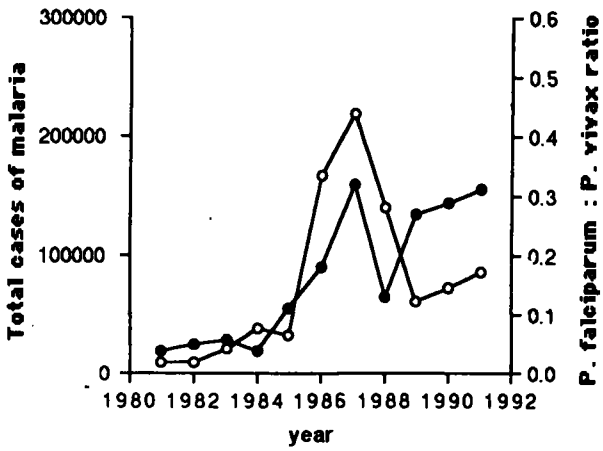

Figure 6. **Pattern of malaria in the study area from 1981 — 1991** (Total number of cases of malaria — open circles, *P. falciparum : P vivax* ratio — closed circles)

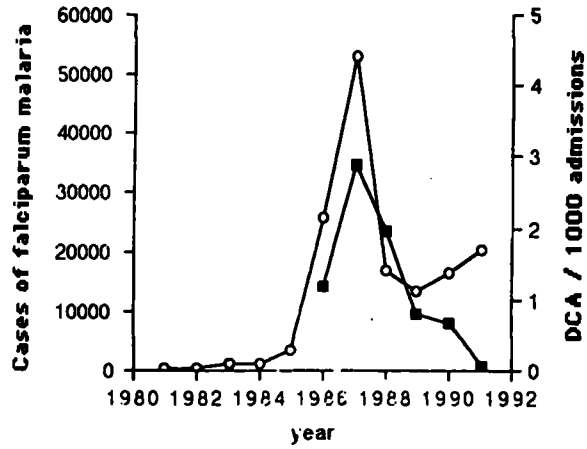

Figure 8. **Relationship between falciparum malaria and DCA** (Cases of falciparum malaria — open circles, incidence of DCA per 1000 hospital admissions — closed squares)

Table 2. Pattern of malaria in the 10 MOH areas surveyed

Year	*No. Falciparum and mixed malaria*	*PF: PV ratio*	*PV: PF ratio*
1981	406	0.04	20.7
1982	449	0.05	19.9
1983	1173	0.05	17.7
1984	1342	0.04	27.4
1985	3281	0.11	8.8
1986	25587	0.18	5.6
1987	53036	0.32	3.6
1988	16922	0.13	7.3
1989	13468	0.27	3.6
1990	16411	0.29	3.5
1991	20217	0.31	3.3

PF - *Plasmodium falciparum*, PV - *Plasmodium vivax*

Table 3. Hospital based survey of delayed cerebellar ataxia (DCA)

Year	*No. of patients with DCA*	*Total hospital admissions*	*Incidence of DCA per 1000 admissions*
1986	33	27902	1.2
1987	85	29588	2.9
1988	55	28085	1.9
1989	21	25958	0.8
1990	19	28182	0.6
1991	02	33779	0.05

Combined data from records of patients admitted during January, February, June and July for each year to Base Hospital Matale and General Hospital Anuradhapura.

The observations on the pattern of incidence of DCA complicating falciparum malaria are based on an epidemiological survey utilizing selective sampling. Although there was no randomness in the sampling, selective sampling is an accepted epidemiological method[45]. This method was chosen because of logistical problems anticipated in attempting to examine the medical records of all hospital admissions during 6 years, and because of the changing monthly incidence of malaria in the region.

The results of this study clearly show that between 1986 and 1991, the incidence of DCA complicating falciparum malaria corresponded closely with the epidemic of falciparum malaria in the region between 1984 and 1988. What is probably even more interesting, was that despite a subsequent increase in falciparum malaria in this area between 1989 and 1991, the incidence of DCA continued to decline, and was near zero in 1991. This suggests that the strain of *P. falciparum* which caused the epidemic between 1984 and 1988, and not the strain or strains responsible for the subsequent increase in falciparum malaria between 1989 and 1991, was specifically responsible for DCA.

The following facts when considered together with the results of this epidemiological survey support the hypothesis that DCA was caused by a new strain of *P. falciparum:*

1. DCA complicating falciparum malaria appeared suddenly for the first time in 1984[23] and there are no previous descriptions of it during past epidemics of malaria or during non-epidemic periods even though falciparum malaria has been endemic in several parts of Sri Lanka for many years.
2. Some of the patients reported to have developed this complication had previously had several attacks of malaria, but had not developed cerebellar dysfunction.
3. The complication occurred in a population who were for the most part residents of areas endemic for malaria (some longterm) and therefore could be considered to have a certain degree of immunity

to malaria. Despite this, they seemed as susceptible to develop this new complication as those who contracted falciparum malaria during a single visit to an endemic area.

4. Except for two case reports[28,29], one of which was very poorly documented and the other where the patient had pancytopenia in addition to cerebellar ataxia, all patients with DCA complicating falciparum malaria have been reported from Sri Lanka. In any event, DCA of epidemic proportions has been seen only in Sri Lanka.

Summary

Cerebellar dysfunction during attacks of falciparum malaria is uncommon, but has been reported to occur since the beginning of this century. There has been a recent resurgence of interest in the cerebellum, following reports of a novel syndrome of delayed onset, selective, cerebellar dysfunction complicating falciparum malaria. Clinical and laboratory evidence exists to suggest that immunological mechanisms play an important role in its pathogenesis. There is epidemiological evidence which supports the hypothesis that this syndrome was probably caused by a new strain of *Plasmodium falciparum*.

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