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Optic Neuropathies in Sub-Saharan Africa

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This thesis is submitted in fulfilment of the requirements

For the Degree of Doctor of Philosophy (PhD)

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Abstract

Purpose: An epidemic of subacute optic neuropathy with sensori-neural hearing loss and peripheral neuropathy has affected Tanzania.

The primary objective of this thesis was:-

To describe the clinical features of the disease.

To describe the magnitude of the epidemic

To investigate the aetiology of the disease

To determine if daily oral B-complex supplements improved prognosis.

Methods: 225 acute cases in Dar es Salaam, Tanzania and 105 acute cases in Mogadishu, Somalia were assessed for signs and symptoms of optic neuropathy, peripheral neuropathy, and hearing loss. Blood and urine samples were collected from very acute cases (< 2 weeks of disease onset) in Tanzania and matched controls for aetiological studies. The same Tanzanian cases were recruited to the treatment study, of which 209 cases came back for 1 month re-assessment and a further 173 patients completed the three month treatment and returned for re-assessment. Ophthalmic, neurological and audiometric examinations were undertaken at baseline and follow-up. Population-based surveys were conducted in Dar es Salaam, Tanzania and Gambia to determine the magnitude of the epidemic.

Results: The optic neuropathy is characterised by bilateral and symmetrical loss of visual acuity and colour vision, and central or caeco-central scotomas. On fundoscopy hyperaemia and swelling of the optic discs were observed at the early stages, as all cases progressed to temporal optic atrophy and nerve fibre layer loss in the papillomacular bundle.

35% of the Tanzanian cases and 59% of the Somalian cases had an accompanying peripheral neuropathy, 65% of the Tanzanian cases and 28% of the Somalian cases had sensori-neural hearing loss.

The prevalence of the epidemic in Dar es Salaam was 2.4%, while in the Gambia it was 0.03%. An estimated 80 000 persons in Dar es Salaam were affected by the epidemic. The disease is concentrated among persons aged 10-39. The poor are particularly affected.

Cyanide intoxication has been ruled out as cause. No evidence of common leber's mitochondrial DNA mutations was found. Breast-feeding is the strongest known risk factor (relative risk of 12.50). There was a deficiency in B-complex vitamins in both cases and healthy controls.

90% of cases who came for re-assessment had substantial recovery of visual acuity, colour vision and hearing.

Conclusions These optic neuropathy cases appear to be identical to Cuban optic neuropathy cases, except that they are younger. A nutritional deficiency appears to be a key component to the epidemic, but is not sufficient by itself to cause the disease. Other unidentified co-factors may be involved. B-complex supplements have resulted in substantial visual recovery for 90% of cases.

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3- Dolin PJ, **Mohamed AA**, Plant GT: Epidemic of bilateral optic neuropathy in Dar es Salaam, Tanzania. N Eng J Med. 1998 May 21; 338(21): 1547-8.

4- **Mohamed AA**, Plant G, Dolin P: Tanzanian optic neuropathy epidemic: outcome following B-complex vitamin supplements. Jeremov conference 97 Oct 19, 4123:148

5- Plant GT, Dolin P, **Mohamed AA**, Mlingi N : Confirmation that neither cyanide intoxication nor mutations commonly associated with Leber's Hereditary Optic Neuropathy are implicated in Tanzanian epidemic Optic Neuropathy. J Neurol Sci. 1997 Nov 6;152(10:107-8.

6- Plant G, Dolin P, **Mohamed AA**: Tanzanian optic neuropathy epidemic: background and clinical features Jeremov conference 97 Oct 19, 4122: 148

The extent of my personal contribution

In accordance with the requirement of University College London, the extent of my personal contribution to the work in this thesis is specified as follows:

During part of the time in which this work was carried out, I was employed as a full time research fellow, on grant from the Welcome Trust (1 year). The grant holder was Dr. Paul Dolin, Epidemiologist at International Centre for Eye Health, the Institute of Ophthalmology , University College London (1996-1997). My primary supervisor was Dr. Gordon T Plant and my secondary supervisor was Dr. Peter Nunn.

I made major contribution to the design of the studies contained in this thesis. I collected all the data, and completed all the analysis.

This thesis is entirely my own original work and no other person should be held accountable for its contents.

Abdirisak Ahmed Mohamed

I certify this is a correct statement of Abdirisak Ahmed Mohamed's contribution.

Dr. Gordon T Plant

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Chapter 1

Optic Neuropathies : Introduction &Literature review

Background :

The anterior visual pathway is susceptible to damage from toxins or nutritional deficiency. These disorders tend to be classified under the heading toxic/nutritional optic neuropathy, a syndrome characterized by papillomacular bundle damage, central or cecocentral scotoma, and reduction of colour vision. Both toxicity and malnutrition, acting either independently or together, have been implicated in the pathogenesis of these disorders. Although these problems have been classified as optic neuropathies, in most of these entities, the primary lesion has not actually been localized to the optic nerve and may possibly originate in the retina, chiasm, or even the optic tracts.

A metabolic lesion is the most likely aetiology of the selective injury affecting neurons requiring high energy consumption. (Sadun et al. 1994) have postulated a mitochondrial impairment of the cytochrome oxidase electron transport chain as a possible mechanism of neuronal injury in Cuban patients. Another alternative is the blockage of the oxidative decarboxylaton of pyruvate and α-ketoglutrate by thiamine deficiency which would prevent the eventual production of acetyl-coenzyme A (acetyl-CoA). Thiamine deficiency also blocks the pentose phosphate pathway by preventing the transketolase reaction. The actual cause(s) of this metabolic lesion could be neurotoxic agents, nutritional deficiencies, genetic defects, or combinations thereof.

1. Nutritional deficiencies: The peripheral neuropathies that manifest as a result of nutritional deficiencies have few individual characteristic signs by which to identify them, but they can be differentiated by observing the other symptoms of the underlying systemic disease.

These deficiencies come in 2 forms, either an isolated deficiency (usually one of the B vitamins) or a complex deficiency resulting from several concurrent metabolic disorders (usually malabsorption).

Pathophysiology: Neuropathies assume 2 forms: (1) definite deficiency (usually B vitamins) or (2) complex deficiency from several concurrent metabolic disorders (usually malabsorption). The mechanisms of the definite deficiencies are as follows:

Thiamine deficiency

Thiamine (vitamin B-1) is found in wheat germs, the outer layer of seeds, nuts, and most vegetables; thiamine pyrophosphate is essential for aldehyde group transfer and is an essential coenzyme for glycolytic and pentose pathways of glucose metabolism. Thiamine is needed by 4 enzymes, pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, transketolase, and branched-chain alpha-ketoacid dehydrogenase. Thirty milligrams (mg) are stored in body tissue, but utilization is about 1-2 mg daily; recommended daily allowance [RDA] for males is 1.5 mg. A deficiency can cause either "wet beriberi," in which congestive heart failure is the primary symptom, or "dry beriberi," in which a peripheral neuropathy is the primary symptom, depending on the percentage of carbohydrates in the diet. Deficiencies preferentially affect the nervous and cardiac tissue because thiamine pyrophosphate is bound less strongly there; daily intake of less than 0.2 mg causes discontinuous degeneration of the axonal sheath with subsequent impairment of the axon, producing a polyneuropathy in about 3 months. The vagal nerve is affected particularly, causing symptoms in the distributions of the cardiac, laryngeal, and recurrent nerves.

Niacin deficiency: Niacin (vitamin B-3) is found in yeast, beef, pork, and chicken. The active form of this coenzyme, nicotinamide adenine dinucleotide (NAD), is essential for electron and acyl-group transfer in glycolysis. A deficiency of niacin causes pellagra.

Pyridoxine deficiency

Pyridoxine (vitamin B-6) occurs widely in plant and animal tissues, such as muscle meat, liver, vegetables, and whole grain cereals; vitamin B-6 consists of pyridoxine, pyridoxal, and pyridoxamine. It is involved in primary carboxylation and transamination, playing a role in metabolizing tryptophan, glycine, serotonin, and glutamate, as well as sulfur-containing amino acids, and it is used in the synthesis of both heme and GABA. Deficiencies usually are associated with increased excretion due to isoniazid ingestion and cause a sensorimotor neuropathy and seizures. Pyridoxine deficiency rarely is associated with a vasculitic mononeuropathy multiplex. The toxic effect of excessive pyridoxine consumed for an extended period of time on the dorsal root ganglions causes a pure sensory neuropathy. A high-protein diet increases pyridoxine requirements, for which the daily requirement for males is 2 mg.

Cyanocobalamin deficiency

Cyanocobalamin (vitamin B-12) is found in meat, especially liver and kidney, cheese, milk, eggs, and fish; this inactive precursor is converted into 2 active metabolites—methylcobalamin and adenosylcobalamin. Methylcobalamin is essential for folate metabolism and the formation of choline-containing phospholipids that are the building blocks of myelin. Adenosylcobalamin is required for the formation of succinyl coenzyme A, the lack of which causes impairment in the formation of neural lipids.

Following ingestion, cyanocobalamin binds with intrinsic factor secreted by parietal cells in the stomach, enabling it to resist proteolysis. Receptors in the distal ileum then facilitate digestion. The liver stores 4 mg of cyanocobalamin, representing a 3-6 year supply; thus, primary deficiencies are rare, except in strict vegetarians and nursing infants, but manifestations of cyanocobalamin deficiency occasionally complicate the presence of malabsorptive disorders. These manifestations, which appear throughout the white matter, are a result of a focal disintegration of medullary sheath known as subacute combined degeneration. The daily requirement for males is 2 mg.

Pantothenic acid deficiency

Almost all foods contain this constituent of coenzyme A, the concentration of which in tissues is 10 times that of thiamine and 50% that of nicotinic acid. Deficiencies are rare because of this large amount of storage, although pantothenic acid has been implicated in the pathogenesis of "burning foot" syndrome. The daily requirements are 6-10 mg.

Alpha-tocopherol deficiency

Alpha-tocopherol (vitamin E) is a lipid-soluble antioxidant, lack of which causes a syndrome resembling spinocerebellar degeneration, reversible in early stages but with devastating consequences if allowed to progress. The daily requirement is 10 international units.

Multi-deficiency factors

This poorly characterized syndrome of neuropathy, visual and auditory deficits is common in prisoner-of-war camp survivors and in tropical countries. The neuropathy is probably due to a deficiency of B vitamins; the sensorineural deafness is postulated to result from deficiency in riboflavin or B-complex vitamins, and the amblyopia, too, may be from a complex deficiency.

2. Toxic factors

Alcohol

Ethanol intercalates into cell membranes, increasing membrane fluidity, but it also affects many signal transduction proteins, including ion channels, secondary messengers, neurotransmitters, neurotransmitter receptors, G proteins, chaperones, and regulators of gene _expression. A peripheral neuropathy is often the earliest symptom of chronic alcohol dependence. Peripheral nerve damage results from the 3 processes (about which considerable debate still exists) of (1) nutritional deficiency, especially thiamine (ethanol interferes with thiamine absorption in the intestine) but also niacin, foliate, and protein; (2) direct toxicity from abnormal products (e.g., phosphatidyl ethanol, fatty acid ethyl esters) and from metabolites (e.g., acetaldehyde that reacts with proteins to form adducts); and (3) indirect toxicity (i.e., neuropathy from hepatic dysfunction).

Cyanide toxicity

Cyanide toxicity from Cassava or smoking has been demonstrated to produce chronic optic neuropathy syndrome in Africa. When prepared incorrectly, cassava can cause cyanide intoxication. Cassava roots, from which several foods are processed, contain cyanogenic compounds that are usually reduced during processing. Cyanogenic compounds, however, often remain in cassava foods because the methods of processing cassava roots are not 100% efficient. Although hydrogen cyanide is rarely detected in these foods, consumption of foods containing cyanogenic compounds has been shown to result in exposure to cyanide, though the amount of cyanide absorbed from a single meal is small.

Cyanide is metabolized to several compounds such as thiocyanate, cyanate, formate, and 2-iminothiazolidine-4-carboxylic acid, but the major metabolite is thiocyanate. Thiols which are sources of sulphur are required to detoxify cyanide to thiocyanate. It has been hypothesized that the detoxification of cyanide to thiocyanate may be impaired in cases of polyneuropathy because of a deficiency of thiols. This metabolism of cyanide to thiocyanate has not, however, been shown to be impaired, even in starvation. This may reflect the continuous release of amino acids from the lean body mass during starvation.

The exact mechanism(s) by which nutrtitional deficits damage the optic nerve has not been elucidated. Although the aetiology is likely multifactorial, most clinicians agree that in patients who abuse ethanol and tobacco, undernutrition is the principal cause of the amblyopia. Others believe that specific deficiencies in vitamin B-12, thiamine, folic acid, or any combination of these also play a role (Glaser 1999).

Why certain agents are toxic to the optic nerve also remains largely un-established.

Whether the unusual configuration of the vascular supply of the optic nerve head predisposes it to the accumulation of toxic agents has been questioned (Lessell S 1994). Toxic and nutritional optic neuropathies resemble each other in terms of their clinical presentation and to most of the optic neuropathies that present simultaneously and bilaterally (Grant 1993).

The anatomical basis of the caeco-central scotoma was first described in autopsy studies of toxic amblyopia, not normal anatomy (Plant et al 1990).

3. Genetic factors

Leber's hereditary optic neuropathy causes a well characterized sub-acute affection of the optic nerves, typically inherited through the maternal line. It is due to mitochondrial dysfunction, usually demonstrated t be a consequence of one of three pathogenic point mutations in the mitochondrial DNA (mDNA). (Sadun AA 2000; Howell n 1997; Chalmers RM 1999). All three of these pathogenic mutations affect complex I in the respiratory chain and the biochemical defect they induce is still unclear. Both an impairment of energy production and/or a chronic increase of reactive oxygen species (ROS) are the potential consequences of the underlying LHON pathogenic mutations leading to optic nerve degeneration (Carelli V et al, 1997; Carelli V et al, 1999).

Leber's hereditary optic neuropathy (LHON)

The discovery that Leber's hereditary optic neuropathy (LHON) segregated in a non-mendelian, maternal pattern [van Senus, 1963; Erickson, 1972; Wallace et al., 1970; Nikoskelainen et al., 1987] suggested a disorder of mitochondrial inheritance [Nikoskelainen et al., 1984a; Egger and Wilson, 1983]. Using a candidate approach, Wallace and co-workers screened the mitochondrial genome from several LHON pedigrees and discovered a point mutation at nucleotide position 11778 responsible for the majority of cases [Wallace et al., 1988, Singh et al., 1989]. Men are more frequently affected with visual loss than women, comprising 80-90% of case series [Newman et al., 1991; Oostra et al., 1994; Riordan- Eva et al., 1995]. Analysis of 85 LHON families demonstrated no statistically significant differences in ratios of affected males to affected females with respect to mutation [Harding et al., 1995]. With the exclusion of index cases and siblings less than 50 years old, the best estimate of recurrence risk is 30% to brothers and 8% to sisters of index cases. Affected females are more likely to have affected children, particularly daughters, than unaffected female carriers [Harding et al., 1995].

The onset of visual loss typically occurs between the ages of 15 and 35 years in most pedigrees. This broad range of ages may occur even within the same pedigree. There are no differences in the age of onset between different mutation groups and between secondary and index cases. The age of onset in females is slightly later than in males [Harding et al., 1995].

Vision loss is, typically painless but may be associated with headache, Uhthoff's symptom (transient worsening with warmth or exercise), eye discomfort, photopsias, limb parasthesias, and dizziness [Newman et al., 1991; Riordan-Eva et al., 1995]. Simultaneous, bilateral vision loss is reported in approximately 50% of cases. In eyes with sequential loss, the average interval to involvement of the second eye is 3 months but may rarely remain monocular up to 16 years of follow-up [Nikoskelainen et al., 1996]. The vision loss typically reaches its nadir within 2 months but may be slowly progressive over a period of greater than 8 weeks. Visual acuity typically deteriorates to worse than 20 /200 but may range from 20/20 to no light perception. Associated with vision loss is progressive red-green dyschromatopsia. Pupillary light responses are relatively preserved in comparison with those in patients with other optic neuropathies [Wakakura and Yokoe 1995].

Visual field defects are typically central or caeco-central absolute scotomas surrounded by a narrow rim of relative scotoma. The classic fundus appearance of circumpapillary telangiectatic microangiopathy, swelling of the nerve fiber layer around the disc, and absence of leakage from the disc on fluorescein angiography [Smith et al., 1973; Nikoskelainen et al., 1982b, 1984b] may be observed in 50% to 60% of affected patients [Newman et al., 1991; Riordan-Eva et al., 1995] as well as in "presymptomatic" cases and asymptomatic maternal relatives [Nikoskelainen et al., 1982a, 1984b].

With time, the hyperaemia and peripapillary nerve fiber layer swelling resolve, leaving temporal pallor and papillomacular nerve fiber layer dropout. Most patients suffer permanent, profound vision loss and do not experience further insults. However, even after a period of stability lasting up to several years, some patients may experience recovery of excellent vision in one or both eyes [Stone et al., 1992). Hearing loss and skeletal abnormalities such as thoracic kyphosis have been reported [Wallace et al., 1970 ; Mackey, 1994; Nikoskelainen et al., 1995]. Minor neurological symptoms may be present in some patients, including tremor, mild cerebellar ataxia, pathologic reflexes and sensory neuropathy [Van Senus, 1963; Wilson, 1963; Funakawa et al., 1995; Nikoskelainen et al., 1995].

While defects in oxidative phosphorylation has been demonstrated by in vivo phosphorus magnetic resonance spectroscopy and in vitro muscle and blood samples [Parker et al., 1989; Larsson et al., 1991; Majander et al., 1991; Toscano et al., 1992; Cortelli et al., 1991], how mitochondrial mutations manifest the LHON phenotype is the focus of ongoing study. No animal models of the disease are available.

In view of the possibility of spontaneous visual recovery in LHON, reports of effective treatment must be interpreted with caution. Present treatment regimens are designed to increase mitochondrial energy production. Agents include naturally occurring cofactors in mitochondrial metabolism and anti-oxidants: coenzyme Q10, succinate, idebenone, vitamin K,, vitamin K3, vitamin C, thiamine, and vitamin B2. Limited initial experience with coenzyme Q and succinate in affected patients has been disappointing. At this point, no therapy has been consistently demonstrated to benefit patients with vision loss or to prevent vision loss among their asymptomatic maternal relatives.

4- Optic neuropathies associated with central nervous system diseases

Optic neuropathy in Human Immunodeficiency Virus (HIV) infection

Visual deterioration in HIV is a common problem. At present, the magnitude of HIV-related neurological complications in sub-Saharan Africa is largely unknown, though at the end of 2001, approximately 28.5 million people were living with HIV/AIDS in this part of the world (UNAIDS, 2003). Ocular manifestations of HIV include retinal microangiopathy, opportunistic retinal infections, Kaposi's sarcoma of the conjunctiva and eyelids, and herpes zoster ophthalmicus. Retinal disease occurs frequently, sometimes in association with optic nerve disease. Occasionally the optic nerve is selectively involved. The commonest aetiology is opportunistic infection. Most case reports have focused on the role of opportunistic infections such as syphilitic optic perineuritis, Cytomegalovirus (CMV) papillitis, varicella zoster optic neuritis, or cryptococcal retrobulbar neuritis (Winward et al 1989).

Other causes include toxoplasmosis and tuberculosis, as they are responsible for the majority of optic neuropathies in HIV patients. The concept of primary HIV optic neuropathy remained controversial until viral genome was isolated in the optic nerves of five patients with axonal degeneration (Sadun et al., 1995). There are two ways in which HIV infection alone may be implicated in optic neuropathy. Firstly, there is now increasing evidence that HIV involves the optic nerve in a chronic process that may not be symptomatically important. HIV was assumed to induce optic neuropathy in 7 patients,, representing 4% of the study population and 7% of patients with neuro-ophthalmologic manifestations (Mwanza et al., 2004). Visual evoked potentials have been shown to be reduced in HIV-positive patients (Malessa et al., 1995).

Morphological studies have shown that the number of retrobulbar nerve fibres in patients with AIDS is decreased compared with normal optic nerves, even in the absence of opportunistic infections (Tenhula et al., 1992). Secondly, an acute optic neuropathy has been described as part of an MS-like syndrome in HIV-positive patients (Berger et al., 1989). Steroid responsiveness may be a feature of HIV-associated optic neuropathy (Burton et al., 1998; Newman et al., 1992). In conclusion HIV may directly cause an optic neuropathy.

Optic neuropathy associated with Multiple Sclerosis (Devic's Disease)

Multiple Sclerosis (MS), originally described as monophasic attack of optic neuritis and myelitis, may pursue a relapsing–remitting course causing severe neurological disability (Wingerchuk *et al.*, 1999). Our first insights into the pathogenesis of multiple sclerosis were obtained following developments in histopathology in the late 19th Century. More than a century later, despite the advanced molecular tools that are now available, significant progress in unravelling the pathogenesis of multiple sclerosis is still very much dependent on similar approaches. The inaccessibility of the CNS for direct study, and the clinical and genetic heterogeneity of multiple sclerosis combine to make the study of multiple sclerosis difficult to say the least (Compston *et al.*, 1998)

Strikingly, all early demyelinating lesions in Multiple Sclerosis are associated with perivascular deposition of immunoglobulins, in particular IgM, local activation of the complement cascade and a marked eosinophilic infiltrate. This combination of features is relatively specific for early NMO lesions, but is also accompanied by immunopathological changes in the CNS such as macrophage/microglia activation and axonal damage that are ubiquitous in all forms of multiple sclerosis.

The immunopathology of MS is highly suggestive of an antibody-dependent, complement-mediated pathogenesis extenuated by the recruitment and degranulation of eosinophils. The lesion distribution also exhibits a preference for the optic tract and spinal cord similar to that seen in MS. .More importantly, the inflammatory infiltrate contains large numbers of eosinophils and demyelination is associated with complement deposition.

Recruitment of eosinophils into the CNS was not seen in demyelinating lesions induced by the co-transfer of MOG-specific antisera and encephalitogenic Th-1 MOG-specific T cells in BN suggesting that eosinophil recruitment was dependent on a MOG-specific Th-2 T cell response. However, it was not possible to identify a clear highly polarized MOG-specific Th-2 T cell response. In particular, no enhanced secretion of the classical Th-2 associated-cytokine IL-4 was observed and the MOG-specific antibody response included both Th-1-associated (IgG2b) and Th-2-associated (IgG1 and IgE) isotypes.

Nevertheless, despite our limitations in understanding the genetics, immunology and cellular biology of MS, the findings of Lucchinetti and colleagues are of substantial therapeutic importance. NMO is associated with clearly defined MRI and CSF abnormalities that should allow us to rapidly identify these patients

Currently it is not possible to identify any of the specific immunopathological features associated with variants of multiple sclerosis such as NMO using non-invasive imaging techniques, although developments in MRI techniques may soon bridge this gap. Once this goal is achieved it will be possible to identify the pathomechanisms driving disease activity and nourishes the hope that 'personalized' therapeutic approaches can be developed for defined multiple sclerosis subtypes.

5- Epidemics of optic neuropathies in the world

The tropics are situated along the equator, between the tropics of Cancer and Capricorn. Poor environmental sanitation, frequent water-borne diseases of viral, bacterial and parasitic origin, ready transmission of respiratory and arthropode-borne diseases, and a high prevalence of nutritional deficiencies are common in the tropics (Roman 1995).

Endemic neuropathies and myleopathies are highly prevalent in the tropics (Roman et al. 1985). In contrast, epidemic outbreaks, characterized by unexpected increase of incident cases over a brief period of time, are relatively uncommon (Schoenberg and Melton 1993).

Forms of ' tropical amblyopia' have been reported previously in people from different parts of the world, including in Afro-Caribbean's in the West Indies themselves. (Strachan, 1888; Madan, 1889; Whitbourne, 1947; Degazon, 1956) and in Afro-Caribbean immigrants to the United Kingdom (Crews, 1963; Owen, 1966; McKenzie and Phillips, 1968; Fasler and Rose, 1980) and the US (Carroll 1971).

In the 1880s, while working as a senior medical officer in Jamaica, Henry Strachan examined "many hundreds" of patients with "multiple neuritis." This group of patients likely consisted of persons with tropical Spastic Paraparesis (TSP), a syndrome predominantly affecting the lower limbs, is currently synonymous with human T-lymphotroic virus type I (HTLV-I) associated mylopathy.

Although Strachan believed that *multiple neuritis* was due to malaria, rather than a nutritional deficiency, (Miller Fisher 1955), later noted that Canadian prisoners of war subjected to malnourishment in Japanese concentration camps during the Second World War developed an ataxic neuropathy with auditory or visual loss similar to those patients described by Strachan. Fisher therefore named this entity Strachan's disease.

The main feature of the Hong Kong POW (prisoner of war) neuropathy were: burning feet, numbness of the feet, hands and face, peripheral oedema, ataxia, impaired vision, sensory deficit for all modalities in the feet, usually diminished reflexes in the lower limbs, Rombergism and optic atrophy. Glosso-stomatitis occurred in most cases.

In 1918, Sir Henry Harold Scott (1872-1956) a past president of the Royal Society of Tropical Medicine and Hygiene described an outbreak of what he termed *central neuritis* in Jamaican sugar cane workers. Scott noted that the patients could be divided into patients with *intestinal symptoms* and others with *nervous symptoms.* Those in the latter group, referred to as the nervous cases, were constipated, a symptom consistent with the clinical syndrome of Tropical Spastic Paraparesis (TSP). Scott description of the patients' gait is consistent with the spastic gait reported in persons with TSP. On fundoscopy, optic atrophy and nerve fibre layer loss in the caeoco-central area are characteristic. Sensory neurological symptoms were noted in a proportion of cases and difficulty walking. The peripheral neuropathy tends to be painful and associated with ataxia when severe. Deafness is a common accompaniment of this syndrome. The occurrence of muco-cutaneous ulcerations in a proportion of cases has suggested a nutritional deficiency.

This syndrome has occurred outside the tropics in prisoners of war (Peraita, 1938). The pathological changes in optic nerve and spinal cord have been described both in cases from the Caribbean (Scott, 1918) and in ex-prisoners of war (Fisher, 1955) and in peripheral nerve biopsies in Cuba (Borrajero et al., 1994).

Tropical Ataxic Neuropathy (TAN)

An ataxic neuropathic syndrome has been described from Tanzania (Haddock et al., 1962), clearly associated with malnutrition in one particular outbreak (Latham, 1964). Several neurological syndromes described from different tropical regions in the past century have been classified as tropical myeloneurpathies and further grouped into tropical ataxic neuropathy (TAN) and tropical spastic paraparesis. (Strachan 1897; Cruickshank e. 1946; Money 1958; Roman 1985).

The syndromes grouped as TAN differ widely in clinical presentation, natural history, and response to treatment. Two of the neurological syndromes grouped as tropical ataxic neuropathy were described in Nigeria in the past 60 years.

In 1930, a syndrome that affected predominantly adolescent school pupils in several boarding schools was reported from some communities in eastern and western parts of Nigeria. (Moore 1930; Moore 1937; Moore 1940). The main clinical features were sore tongue, angular stomatitis, and skin desquamations followed by optic atrophy in large proportion of cases. Recovery of the lesions occurred during holidays, but many affected pupils had a recurrence on returning to school. (Moore 1939).

The school diet, dominated by cassava food products, was implicated as causal and almost 100% of the patients responded to improvement of diet and supplements with autoclaved dried yeast.(Moore 1937). Moore considered the disorder to result from vitamin B complex deficiency, but the syndrome of optic neuropathy, deafness and peripheral neuropathy occurring in the tropics has never been shown to result from a specific micronutrient deficiency, as is the case in the neurological syndromes accompanying pellagra and beriberi, for example. (Moore 1939).

The second syndrome, unlike the syndrome of the 1930s, affected middle aged and elderly people predominantly and its geographical distribution was limited to some communities in southwestern Nigeria. (Money 1958; Money 1959).

It was variously named endemic neuropathy (Money 1958), degenerative neuropathy (Osuntokun et al. 1969), tropical nutrition neuropathy (Osuntokun 1971) and tropical ataxic neuropathy (Osuntokun 1968). Different combinations of sensory polyneuropathy, bilateral optic atrophy, bilateral neurosensory deafness, and sensory gait ataxia were found. Its causation was attributed to dietary cyanide from the monotonous consumption of food (Monekosso 1964; Osuntokun 1981) processed from the starchy roots of cassava (*Manihot esculenta esculenta).* In contradistinction to the first syndrome, cases did not respond to improvement in diet or vitamin supplementation (Osuntokun 1970; Osuntokun 1974).

In the 1980s, the occurrence of TAN was reported to have subsided because cases were no longer registered in the teaching hospitals. It was speculated that improvement in diet, which followed the oil boom economic recovery period of the 1970s in Nigeria, caused the disappearance of the syndrome (Osuntokun 1994). However, a recent food survey in Ososo, a semiurban community of Ijebu speaking Yorubas in south-western Nigeria previously surveyed in 1969 (Osuntokun 1969), showed that about 80% of the population consumed foods processed from the starchy roots of cassava thrice daily (Onabolu 1999). TAN remains endemic in Ososa, Nigeria, and it seems distinct from Strachan's syndrome, epidemic optic neuropathy in Cuba and Tanzania, and Konzo. However it shares feature with a subset of cases described from prisoner of war camps ((Oluwole et al., 2000).

Tropical spastic paraparesis ; A condition characterized as a 'tropical' spastic Paraparesis, with minimal sensory deficit, was described first from Zaire, where it is known as 'Buka-buka, Kitondji or Konzo' in local languages (Carton et al., 1986). Acute as well as chronic spastic paraparesis of unknown origin is much more common in tropical countries than in temperate climates. Deficiencies or toxicities due to primitive diets as well as infectious agents have been implicated (Spillane 1973).Sometimes these diseases occur in epidemics. The earliest documented cases of epidemic spastic paraparesis in the Bandundu region of Zaire occurred in 1928, 1932 and 1937 (Trolli 1938; Lucasse 1952).

Ever since there have been new outbreaks affecting hundreds of cases in several areas of bandundu, the latest ones in 1978 and 1981. One of the latest outbreaks was observed in 1981 in Masi-Manimba affecting 200 women and children (Anonymous 1982). Between epidemics, there have been new sporadic cases every year. For example, since a first upsurge in the area of Kimbongo in 1978 leading to hospitalisation of 67 patients, five to seven new cases are seen each year at the Hôpital/Dispensaire. In Zaire the disease appears to be restricted to the administrative region of Bandundu but it is also rampant in the neighbouring northern part of Angola and in a drought-affected area in Mozambique (Ministry of Health, Mozambique 1984a).

The disease is characterised by a sudden onset of bilateral spastic paresis of the lower limbs, often associated with speech disorders and visual complaints. Recovery may be complete but some patients are left with a typical spastic gait, and more rarely with dysarthria.

The clinical picture is conspicuously uniform. Without preceding illness or prodromal symptoms, the patients start complaining of painful paraesthesiae in the legs, and within a few hours or days develop weakness of the lower limbs, often with visual complaints consisting mainly of visual impairment, temporal pallor of the optic disc. There are visual field defects, the most frequent being concentric constriction and peripheral defects. No correlation was found between the severity of the motor disability of Konzo and the extent of visual field loss. In conclusion, Konzo is associated with optic neuropathy and few patients had pendular nystagmus in primary gaze.

The optic neuropathy in Konzo is thought to be due to cyanide toxicity, and it does not however, resemble the features of the epidemic optic neuropathy in Tanzania, Cuba or Nigeria, Leber's hereditary optic neuropathy, tobacco amblyopia or vitamin B deficiency.(Mwanza et al., 2003). In a study of visual evoked potentials in Konzo patients, it was found that there were delayed P100 latency and decreased amplitude. These findings indicated presence of axonal loss in the prechiasmal visual pathways in Konzo (Mwanza et al 2004)

This form of spastic paraparesis differs but shows similarities with other forms of myeloneuropathies reported from populations in tropical countries (Cruickshank, 1976; Roman et al., 1985). Lathyrism, is the tropical myelopathy most similar to Konzo, except for the absence of visual disturbances. It is characterized by the same acute onset of permanent selective damage to the upper motor neurons affecting the same age group in epidemics during food shortage periods. Lathyrism is caused by high consumption of the drought-resistant chickling pea (*lathyrus sativus)*, a crop that does not exist in any of the areas affected by konzo, but consumed in parts of Ethiopia, India and Bangladesh (Ludolph et al., 1987).

The overall similarity between lathyrism and konzo suggests a similar or common final pathogenic mechanism. Recent experimental work has shown that the excitatory amino acid beta-N-oxalylamino-L-alanine (BOAA) found in the chickling pea and having chemical relationship to the amino acid beta-N-methylamino-L-alanine (L-BMMA) is likely to be causally associated with lathyrism (Spencer et al., 1986).

This disorder has been convincingly shown to be related to subacute cyanide toxicity from poorly processed cassava in the diet. This sustained high blood cyanide concentrations are maintained by a deficient sulphur intake, impairing cyanide to thiocyanate conversion (Tylleskar et al., 1991; Tylleskar et al., 1992). Identical cases have been reported from Mozambique (Casadei et al., 1990) and Tanzania (Howlett et al., 1990) where it has been associated with optic neuropathy -- 8 of 24 cases had visual loss and 6 had temporal optic atrophy (Milingi et al., 1991). This condition is now clearly distinguished from human T-cell leukaemia virus-1 (HTLV-1) associated myelopathy (Rodgers-Johnson et al., 1988), which is also a cause of spastic paraparesis in the tropics.

Cuban Epidemic Optic Neuropathy

Introduction

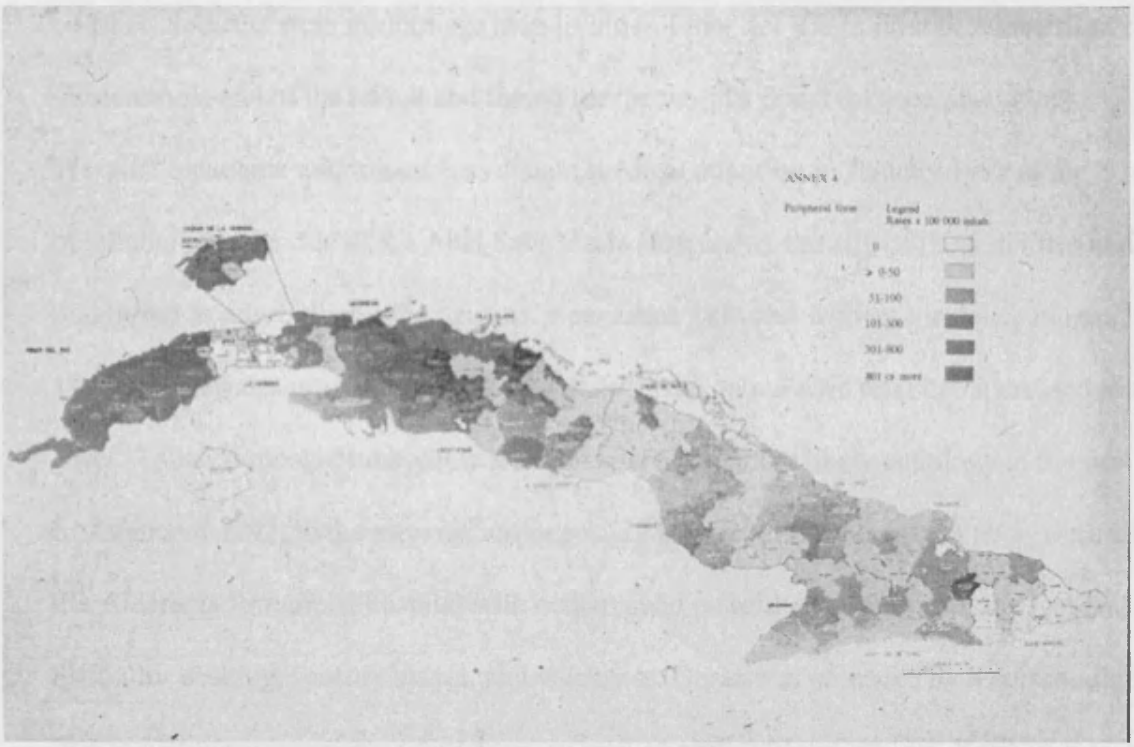


Fig 1.1: The map of Cuba: (Prevalence of Optic Neuropathy): Pinar del Rio province in the west recorded the highest prevalence, and Guantanamo in the east had the lowest prevalence (Mission to Cuba PAHO/WHO 1993).

Cuba: Population is 11 million; land area is 111.000 sq km. The government continues to balance the need for economic loosening against a desire for firm political control. The government reluctantly allows a large dollar market sector, fuelled by tourism and remittances from Cubans abroad. In 1991, with the collapse of the Soviet Union, Cuba has lost the Soviet economic aid which was bankrolling the whole economy, resulting in an economic depression. The loss of the Soviet economic aid, combined with the US complete embargo to Cuba has led to a large section of the population being susceptible to nutritional disorders.

Cuba recently endured an epidemic of neuropathy that resulted in more than 50 000 patients (Mission to Cuba PAHO/WHO 1993). The index cases first noticed loss of vision in November of 1991, Subjects were middle-age men living in Pinar del Río, a rural province situated at the westernmost end of the island and famed for the world's finest tobacco plantations.

The first 8 patients with visual loss sought medical attention in January 1992 at the ophthalmology service of the Abel Sant María Hospital in the city of Pinar del Río and were diagnosed as retrobulbar optic neuritis. New cases followed with an incidence ranging between 14 and 36 new cases per month. By July of 1992 the cumulative total had increased to 168 cases. Tobacco-alcohol amblyopia was considered the most likely aetiology in the first cases.

In August of 1992, in the city of Cienfuegos, 22 inmates from the Ariza Prison were admitted to the Alderegía Provincial hospital with oedema and painful dysesthesias of the feet and legs, difficulty walking, sensory ataxia, and weakness. Onset was preceded by recurrent diarrhoea due to giardiasis or amoebic dysentery, as well as profound weight loss. Beriberi was suspected and patients responded to treatment with thiamine, B-group vitamins and appropriate diet. (Espinosa et al., 1993).

At the end of the first year 472 patients had been reported from 6 of 14 provinces of Cuba: Pinar del Río, la Habana, Cienfuegos, Holgun, Sancti Spritus and Santiago del Cuba. Three months later the number had escalated to 4461 cases. On March 20, 1993 a task Force coordinated by the Civil Defence for Disaster Relief, the Ministry of Public Health and the Cuban Academy for Sciences was organized to confront the growing problem. The President of the State Council, Fidel Castro, chaired this group. 6 research groups were formed: epidemiology, toxicology, basic sciences, nutrition, clinico-therapuetic, and food resources.

Between March and April 1993 an island-wide initiative was launched to increase case ascertainment and to promote early treatment.

Approximately 18 000 family doctors of Cuba's unique community-based primary health care system (Teja Pérez et al., 1992) were employed for this effort. Each family doctor cares for about 100 families and this initiative resulted in a 40% surge of incident cases increasing the cumulative total to 11797 cases (Ramírez et al., 1993). The number of reported cases of peripheral neuropathy continued to increase. These occurred either as isolated neuropathies, or associated with optic neuropathy, deafness, or both. Also during April of 1993, patients with prominent ataxia and pyramidal involvement were admitted to the Saturnino Lora hospital in the city of Santiago de Cuba. A dorsolateral myeolpathy, probably related to vitamin B_{12} deficiency, was suspected (Estrada Acosta et al. 1993). During March, April and may of 1993 the epidemic curve reached an irregular plateau with a cumulative total of 45 584 cases.

Finally , by early June a precipitous drop in incidence occurred. A temporal association was noted between the decline in cases and the distribution to the entire population of Cuba of a vitamin supplement containing B-group vitamins, folate, and vitamin A. Distribution began in the first week of May, 1993. During the last semester of 1993 incident cases gradually abated and by the year's end the epidemic vanished. The total number of cases officially tallied at 50 862 of January 14 1994.

At the closing of the 32nd epidemiological week (7 August 1993) a total of 50 253 cases had been reported for an overall cumulative incidence rate (CIR) per 100 000 inhabitants of 461.64 for all forms of the disease. According to the preponderance of visual or peripheral nerve symptoms, cases were classified for epidemiological purposes as optic forms (CIR 242.39) or peripheral forms (CIR 219.25). However, combined forms were also quite common.

Age distribution showed minimal number of cases in adolescents and children younger than 15 years of age, in pregnant women, and elderly people age 65 and older. Most cases (87%) occurred between ages 25 and 64 years. Optic forms predominated in the age group 45 to 64 years, being slightly more common in men than in women. In contrast, peripheral forms occurred most commonly in women between 25 and 44 years of age. Overall the large majority of patients (93%) lived in urban areas and there was a slight preponderance of men (52%). There were no fatalities attributable to the epidemic neuropathy.

The geographic distribution showed a west-to-east pattern of decreasing incidence with very high rates in Pinar del Río and few cases in Guantanamo. Evidence of contact was low among patients and there was no evidence of contagion (Mission to Cuba PAHO/WHO 1993). A number of case-series and case-control studies were performed by the Cuban Task Force Epidemiology Group (Grupo Operative Nacional 1993).

A study of 4022 patients from Pinar del Río was interviewed to define their social, occupational and demographic characteristics. The patient's history of toxic exposure, nutritional history, as well as possible predisposing factors were also reviewed. The main finding was a strong association of the optic form to smoking (74%) and weight loss (79%).

Furthermore, up to 81% of the patients with peripheral form and 85% of those with combined forms mentioned weight loss preceding the onset of symptoms. Additionally, case-control studies including 708 age- and sex-matched pairs nationwide were performed (Instituto Nacional de Higiene 1993). Distribution by age, sex, and clinical form in these samples reflected national trends.

These studies confirmed the increased risk of disease development in those with smoking history (odds ratio (OR) 4.9 and 95% confidence interval (CI) 2.5-9.3), history of irregular diet consumption (missing one or more meals per day with lower intake of animal protein, fat, and vitamin B-rich foods) (OR 4.7 (2.5-8.8), combined smoking and drinking history (OR 3.5 (1.7-7.4). weight loss (OR2.8 (2.2-3.6); and heavy drinking (OR 2.3 (1.0-5.4)) (Mas Bermejo and colleagues 1993). Pesticide exposure and contact in the same household with persons affected by the disease carried no increased risk (Mas Bermejo et al. 1993).

Clinical Aspects: A wide variety of clinical features were observed in the course of the epidemic including the following: (1) optic neuropathy, (2) sensory peripheral neuropathy, (3) dysautonomic neuropathy, (4) dorsolateral myeloneuropathy, (5) sensorineural deafness, (6) dysphonia and dysphagia, (7) spastic paraparesis, and (80 mixed forms.

Inclusion criteria admitted as "suspected case" any person consulting in Cuba during 1992 and 1993 because of visual symptoms or peripheral sensory symptoms, or both, with subacute onset (between 3 and 30 days) and usually in association with weight loss, lack of energy and easy fatigability (IPK 1993). Diagnostic evaluation and confirmatory tests were performed by teams of experts located in 60 diagnostic centres in all the provinces.

Teams included internists, ophthalmologists and neurologists equipped with ophthalmoloscope instruments, tangent screens for visual testing, Ishihara plates, contrast sensitivity tests, 128-cycle tuning forks, and clinical neurophysiology equipment for nerve conduction studies and evoked responses.

Confirmed cases were hospitalised for treatment with intravenous B-group vitamins and folic acid. In addition to this basic therapy other forms of treatment were explored. Less than0.1% of the patients were left with moderate to severe sequelae.

Investigation, diagnosis and treatment of this large-scale patient load required mobilization of Cuba's already limited resources, including a 30% increase in the number of hospital beds, urgent importation and air transportation of medications and supplies, training of physicians, production of clinical neurophysiology equipment, and purchase (against major difficulties imposed by the economic embargo) of reagents and materials for research laboratories in Cuba.

Optic neuropathy

Patients referred subacute and slowly progressive loss of vision in both eyes described as blurred vision, accompanied by photophobia with increased sensitivity to sunlight, dyschromatopsia or loss of red-green colour vision, and less frequently burning eyes, lacrimation and retro-ocular pain. Visual acuity was decreased bilaterally and usually symmetrically, ranging from moderate (20/80) to severe loss (5/400). Perception of Ishihara colour plates ranged from 7 of 8 to nil. Contrast sensitivity tests demonstrated losses to high and mid spatial frequencies in moderate cases and across all spatial frequencies in severe cases. Bilateral, more or less symmetric, central or caeoco-central scotomata were evident with the Amsler grid test, or more clearly with the threshold Amsler test using cross-polarizing lenses (Wall and Sadun 1986).Tangent field-testing with a white target revealed normal peripheral fields and confirmed the presence of central scotomata (up to 5 degrees from fixation), often revealing caeco-central scotomata. Minimal afferent papillary defects were seen and except for saccadic pursuits ocular motility was usually normal. Fundus examination revealed normal optic discs in most cases. About 12% of the cases presented slight hyperaemia of the optic nerve heads and capillary tortousity (Santiesteban et al. 1993). A typical finding in Cuban cases, was the presence of a wedge-shaped loss of fibres of the maculopapillary bundle (MPB), often with swelling of the nerve fibre layer above and below the MPB (Sadun 1994).

Although observed in other conditions, this finding was considered pathognomonic in the context of the Cuban epidemic. In advanced cases, temporal optic disc pallor occurred (Santiesteban et al. 1993); Lincoff et al. 1993; Sadun et al. 1994).

Visual evoked responses (VERs) revealed moderate slowing of the p100 wave in two-thirds of the patients, mainly in those with severe visual deficits. Significant interocular differences in latencies were noted in half of the patients with abnormal VERs (Santiesteban et al. 1993; Serrano et al. 1993). In a group of 602 patients with optic neuropathy studied by Santiesteban and colleagues (1993), about one-third presented with skin and mucous membranes lesions consistent with undernutrition, 32% had evidence of concomitant myeloneurpathy and 21% had hearing deficits demonstrated by audiometry.

Marked improvement of visual acuity and colour vision was accomplished in the large majority of patients following intravenous treatment with vitamin B complex and folic acid. Less satisfactory results were obtained in patients with long delays from first visual symptoms to vitamin treatment.

Peripheral neuropathy

Patients presented with complaints of painful dysesthesias involving the soles of the feet, less often also the hands, and rarely the face, the lips and the perioral region. Symptoms were described as "burning feet", "hot vapors", numbness, cramps in the feet and calves, tingling and pins-and -needles (Carbera et al. 1993; Pérez 1993). Often, areas of hyperesthesia were noted on the feet and legs; true hyperathia was present predominating at night and interfering with sleep. Nerves were painful to palpation and patients complained of paresthesias in certain positions (pressure points) and of lancinating pains evoking those of tabes (Pérez lache 1993).

In general, there was no motor paralysis but most patients complained of easy fatigability and muscle pains with exercise. Instances of bilateral foot drop with stoppage, as well as rare cases of paralysis of hand dorsiflexors, were seen.

These cases could have been caused by compression of lateral peroneal and radial nerves at pressure points due to weight loss and increased nerve sensitivity to pressure. Nonetheless, they could also represent true cases of a sensorimotor neuropathy such as beriberi. Often, neurological examination disclosed relatively mild objective findings compared with the severity of the subjective complaints. Patients usually had decrease or loss of vibratory perception distally in the limbs, and some also had decreased perception of soft touch, pinprick and temperature in a "stockings and gloves" distribution.

Tendon reflexes were lost or decreased distally. Varying degrees of muscle weakness could be demonstrated distally in the legs, but in general there was no obvious muscle wasting or paralysis. Motor-nerve conduction velocities were normal and sural sensory-nerve potentials were decreased only in severe cases. Sural nerve biopsies showed no axonal neuropathy affecting predominantly large-diameter myelinated axons (Borrajero et al. 1994).

Dorsolateral myeloneuropathy

These patients presented with sensory complaints similar to those described above. However, there was also an increase in urinary frequency, impotence in males, weakness of the legs and difficulty walking. Nocturia, urgency, and involuntary micturition were common but incontinence or urinary retention was exceptional.

Examination disclosed loss or significant decrease of vibratory perception in the feet, with decreased position sense, unsteadiness on Romberg test and sensory ataxia in severe cases. Gait alterations and frequent falls were often present.

Reflexes were brisk in the knees with crossed-adductor responses, contracting with decreased ankle reflexes. Spasticity and Babinski signs were usually absent. Proximal motor weakness was present in approximately one third of the patients. A few patients developed spastic paraparesis. Somatosensory evoked responses with tibial nerve stimulation showed mild delay of the p40 wave (Pérez et al. 1993).

Sensorineural deafness

Patients presented with complaints of hearing loss usually in association with peripheral sensory symptoms, visual loss, or both. Tinnitus, usually of high pitch (5-30 db, 4-8kHz) was a common accompaniment (Arias and Echemendia 1993). The deficit was subclinical in about 25% of the patients with proven loss of hearing by audiometry.

Pure tone audiometry demonstrated high frequency (4-8 kHz0) hearing loss bilateral and usually symmetrical. Abnormal brainstem auditory evoked responses were seen in 35% of the patients with abnormal audiometry (Serrano et al. 1993).

There were no associated vestibular symptoms in most patients. Although nystagmus was present in a few instances. Acoustic trauma and ototoxic exposure were ruled out. The pattern of sensorineural deafness was similar to that of presbycusis (Suga and Lindsay 1976), although young adults were affected, often with severe sensorineural deafness. In general, there was minimal return of hearing in response to treatment.

Mixed forms

In a comprehensive analysis of 356 patients studied in Pinar del Río by Serrano and colleagues (1993) the following distribution of clinical forms was found: pure optic neuropathy (45%), optic neuropathy and myeloneurpathy (24%), optic neuropathy with hearing loss (14%), peripheral and optic neuropathy with deafness (7%).

Other signs and symptoms

In addition to weight loss and lack of appetite, many patients complained of symptoms reminiscent of the chronic fatigue syndrome, with loss of energy, and difficulties with concentration and memory. Some patients presented dysautonomic symptoms, mainly a sensation of body heat accompanied by excessive sweating, coldness, and hyperihidrosis of hands and feet. Palpitations, occurring either spontaneously or following minimal efforts, were relatively common (Pérez Lache 1993).

Overall, there was no evidence of infection or inflammation by clinical or laboratory parameters. Cerebrospinal fluid (CSF) examinations disclosed in some cases mild alterations of the blood-brain barrier (Alfaro et al. 1994) but were otherwise normal. There were no consistent abnormalities of electrolytes, endocrine, haematological, renal or liver function tests.

Aetiology: Nutritional Deprivation

This epidemic disease was characterized by selective involvement of just a few neuronal groups and axons, including the maculopapillary bundle; axons in the dorsal columns (fasciculus gracilis); and concurrent distal axonal degeneration of large myelinated peripheral sensory axons, high frequency spiral ganglion neurons of the cochlea, and distal-most pyramidal tract fibres. A metabolic lesion is the most likely cause of this selective injury, affecting neurons requiring high energy consumption.

Numerous neurotoxins, such as agents capable of producing amblyopia or scotomata, were excluded. In particular, methyl alcohol, organophosphate pesticides, trichloroethlyene, and chronic cyanide intoxication from cassava in the diet were carefully excluded. A Coxsackievirus was isolated in Cuban laboratories, but this could not be confirmed at the National Institutes of Health or two other laboratories in the United States.

Genetic predisposition, in particular, mitochondrial DNA point mutations associated with Leber disease, was excluded in the group of patiens from Pinar del Río (Newman et al. 1994).
The Nutritional origin of the epidemic appears inescapable: Early treatment of patients with B group vitamins produced complete remission. Distribution of vitamins to the entire population of 11 million Cubans rapidly curbed the epidemic (Ministry of Health, Cuba 1994; Roman 1994).

Tanzanian Epidemic Optic Neuropathy

The ophthalmologic features of the disorder consisted of bilateral, simultaneous, usually painless, visual loss, which is progressive over a period of 2-12 weeks. There is a predominantly tritan loss of colour vision and symmetrical central or caeoco-central scotomas. On fundoscopy, few of these patients have been seen acutely, when either a normal optic disc or a swollen hyperaemic disc is seen. In some the macula was described as 'oedematous'.
After four weeks, there appeared to be pigment epithelial changes in the form of mottling, stipppppling or clumping beneath the macula. However fluorescein angiograms did not show any window defect or other abnormality in the retinal pigment epithelium. Optic atrophy and nerve fibre layer loss in the caeco-central area are characteristic (Plant et al 1997). There is some evidence of spontaneous partial recovery of vision if cases are followed for one year. A preliminary ophthalmologic investigation in Dar es Salaam in 1990 suggested that, although the optic nerve was involved in most cases, a maculopathy was present in some (Johnson 1993). Neurological symptoms were burning dysaethesiae and painful feet in the majority, with some complaining of sensory disturbance (numbness, tingling), which was painless. Nerve conduction studies showed evidence of a sensory axonal neuropathy with involvement of large fibres. Motor conduction studies were normal (Plant et al 1997).

Sensorineural deafness, with loss particularly at lower frequencies was confirmed in all cases with symptoms of hearing loss. No patient had oscillopsia or any symptoms which might suggest severe vestibular failure and the vestibulo-ocular reflex was not impaired on clinical testing (Plant et al 1997). Throughout the tropics, there are several myeloneuropathies that occur in geographic clusters (Roman 1985).

The two main forms of tropical myeloneuropathies (TAN and TSP) are known to have occurred in Tanzania. A distinct upper motor neurone disease has been observed in two remote rural areas (Howlett et al 1990; Mlingi et al 1991).

This appears to be identical to the condition "Konzo" described originally from Zaire, and also from neighbouring Mozambique. Of 42 patients with spastic paraparesis in the reported series from Tanzania, visual loss was noted in 8 cases, pallor of the temporal optic disc was observed in 6 cases, and none had hearing deficit. Seven cases of an ataxic neurological syndrome in Tanganyika were described in 1962, believed to be the first reported in East Africa (Haddock et al 1962). Reduced visual acuity was recorded in 3 and pallor of the optic discs in 4. The main feature was gross ataxia. A similar ataxic syndrome, without optic atrophy, was reported in 8 prisoners (Latham 1964). Biochemical evidence in support of chronic cyanide toxicity was obtained in 8 patients with ataxic tropical neuropathy attending Muhimbili Hospital (Makene et al 1972). It is not clear how many of these had optic atrophy.

Although 8% of Dar Es Salaam's population are from Asian or European origin, all cases of Tanzanian epidemic optic neuropathy have been observed in Africans. There is a tendency for the cases to be more common in the lower economy housing area, but not exclusively. There is no clustering by place of occupation. It is not clustered in families as would be expected from a water-borne infection or toxin (Plant et al 1997).

The ocular findings, and the associated peripheral neuropathy, are essentially identical to the Cuban epidemic. The age distribution in the Tanzanian epidemic, however, is distinct as a younger age group has been affected than in the Cuban epidemic. Serological testing for syphilis has been negative. HIV testing has shown the same proportion of positives as in the general population (Johnson et al., 1994). There were negative results for HTLV-1 infection (Plant et al 1997).

Table 1.1 Comparison between different optic neuropathies (Mwanza PhD thesis 2004)

	Konzo	Tropical Ataxic neuropathy	Tanzanian /Somalian Epidemic Optic neuropathy	Cuban Epidemic Optic Neuropathy	Vitamin B1-B12 deficiencies	Tobacco Amblyopia	Leber's hereditary op neuropathy
Onset age	3-35	40-50	10-40	25-45	No predilection	45+	15-35
Onset type	Acute	gradual	Acute	Acute	Acute/gradual	Gradual	Gradual
Course	Non progressive	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive
Type	Irreversible	Irreversible	Reversible	Reversible	Reversible	Reversible	Irreversible
Vision	Blurred vision at onset	Severe bilateral loss	Severe bilateral loss	Bilateral loss	Bilateral loss	Bilateral loss	Severe bilateral loss
Optic disc	Temporal atrophy	Complete atrophy	Temporal atrophy	Temporal atrophy	Atrophy	Temp.atrophy	Atrophy
V. fields	Concentrical constriction	Periph.constriction	Cecocentral scotoma	Cecocentral/central scot.	Cecocentral scot	Cecocentral scot.	Central scot.
Colour V.	Impaired	?	Impaired	Impaired	Impaired	Impaired	Impaired

Periph.Neu.	No	Yes	Yes	Yes	Yes	No	No
S deafness	No	Yes	Yes	Yes	No	No	No
Other signs	Spastic paraparesis	Ataxic gait,absence of tendon reflexes	Ataxic gait in some patients	Decreased tendon reflexes	Depend on the deficient vitamin	None	Neurologic, cardiac sequaelae
Aetiology	Cyanogenes in cassava?	Cyanogens in cassava?	Unknown	B-vitamin deficiencies (+ cyanide from smoke) ?	B-vitamin deficiencies	Cyanide from smoke (+ nutritional, B12 deficiencies) ?	Genetic mitochondrial dysfunction (+ cyanide exposure)
Reference	(Mwanza et al, 2003)	Osuntokun et al, 1971)	(Plant et al., 1997)	Roman, 1994)	Gill and Bell, 1982)	Solberg et al., 1998)	Huponen,2001)

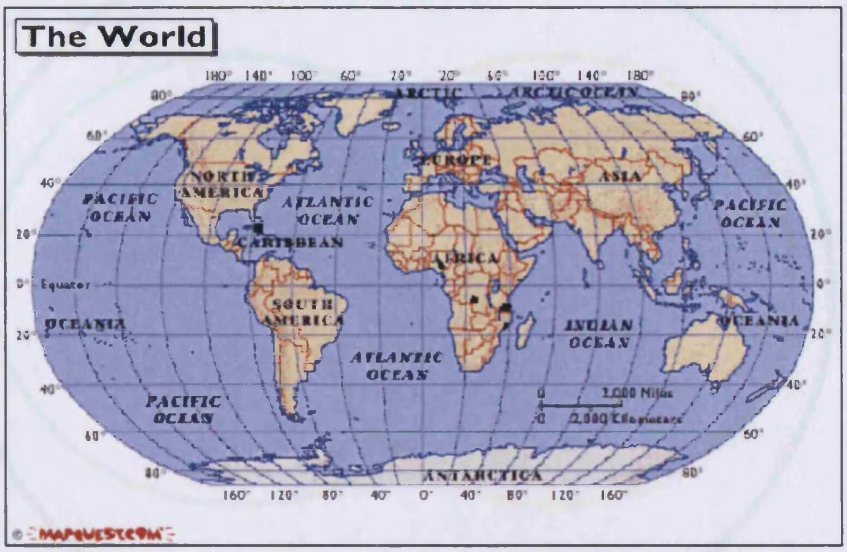


Fig 1.2: The world map with areas of recorded neuropathy epidemics (Cuba, Tanzania, Mozambique, Zaire, Nigeria)

This map shows the areas where epidemics of optic neuropathies, were reported. This apparent distribution might be the result of under-reporting, and the disease may be occurring in other areas of the world.

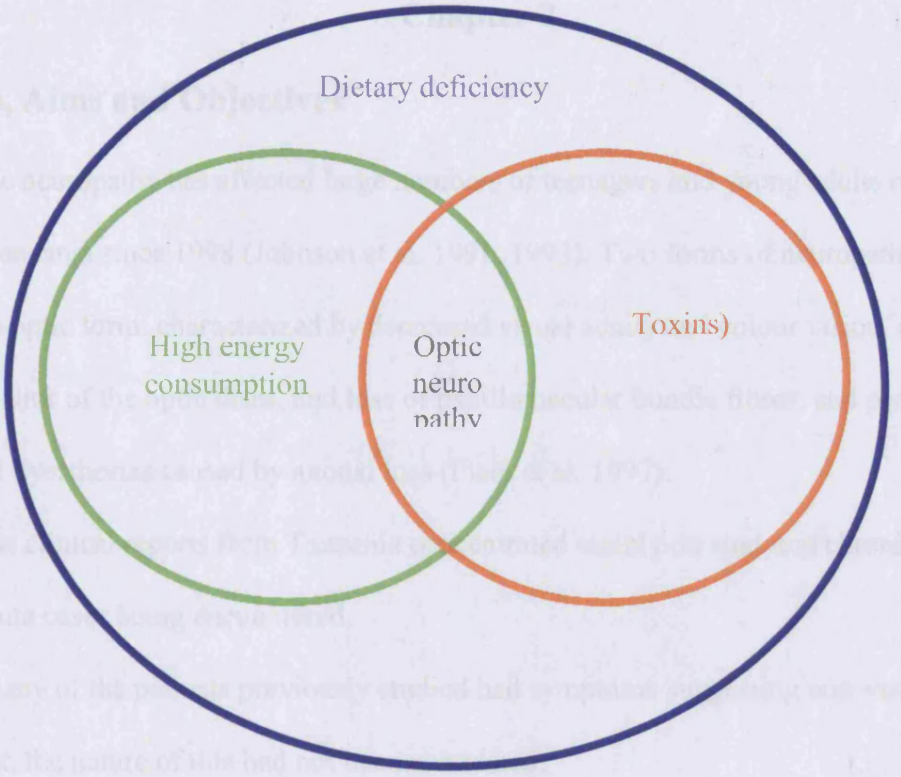


Fig 1.3: Possible inter-relations of risk factors to optic neuropathy

In all communities where the epidemic was observed, a background dietary deficiency was present in cases and controls. We think high energy consumption, mainly by young adults and/or possible dietary toxins are responsible for the disease to affect sections of the community.

Chapter 2

Rationale, Aims and Objectives

An epidemic neuropathy has affected large numbers of teenagers and young adults of African origin in coastal Tanzania since 1998 (Johnson et al. 1991, 1993). Two forms of neuropathy were reported: an optic form, characterized by decreased visual acuity and colour vision, central scotomas, pallor of the optic discs, and loss of papillomacular bundle fibres; and peripheral form, with painful dyesthesias caused by axonal loss (Plant et al. 1997).

The previous clinical reports from Tanzania concentrated mainly on studying chronic cases, with very few acute cases being encountered.

Although many of the patients previously studied had symptoms suggesting non-visual neurological involvement, the nature of this had not been elucidated.

Previous investigations have failed to establish aetiology (Johnson et al., 1994). Serological testing for syphilis has been negative. HIV testing has shown the same proportion of positives as I the general population. There were also negative results for HTLV-1 infection (Plant et al., 1997).

The aetiology of this condition is as yet unknown, although the pattern of occurrence amongst those with poor nutrition had led to the belief that dietary factors play an important role. Furthermore, Cuban epidemic optic neuropathy cases demonstrated marked and almost dramatic recoveries, especially of visual acuity and colour vision following vitamin B complex supplements. The epidemic in Cuba has been essentially stopped by prophylaxis with vitamin B complex distributed throughout the country.

The aim of the present thesis was:

1- To determine the magnitude of the epidemic in Tanzania and the Gambia.

2- To characterize more precisely the extent of the optic nerve and retina damage in the acute stages of the disease and their clinical presentation.

3-To carry out neurological examination in the present group all of whom presented with visual symptoms. The incidence and nature of the neurological involvement will assist in relating this epidemic to the various reports of optic neuropathy, peripheral neuropathy and myelopathy occurring in the tropics.

4-To ascertain etiological causes implicated in the Tanzanian optic neuropathy epidemic.

5- To determine if treating acute cases with daily oral B-complex supplements for 3 months improved their visual, neurological and auditory prognosis.

Chapter 3

Methods

The studies described in this thesis, are based on data from field, clinical, and laboratory studies conducted in Tanzania, the Gambia and Somalia.

Table 3.1: Study types and their locations

Country	Tanzania	Gambia	Somalia
District	Dar Es Salaam	Whole country	Mogadishu
Year	1996-1997	1996	2002-2003
Prevalence study	Yes	Yes	No
Case series	Yes	No	Yes
Etiological study	Yes	No	No
Treatment study	Yes	No	No

Background Information: Fig 3.1 Tanzanian Map

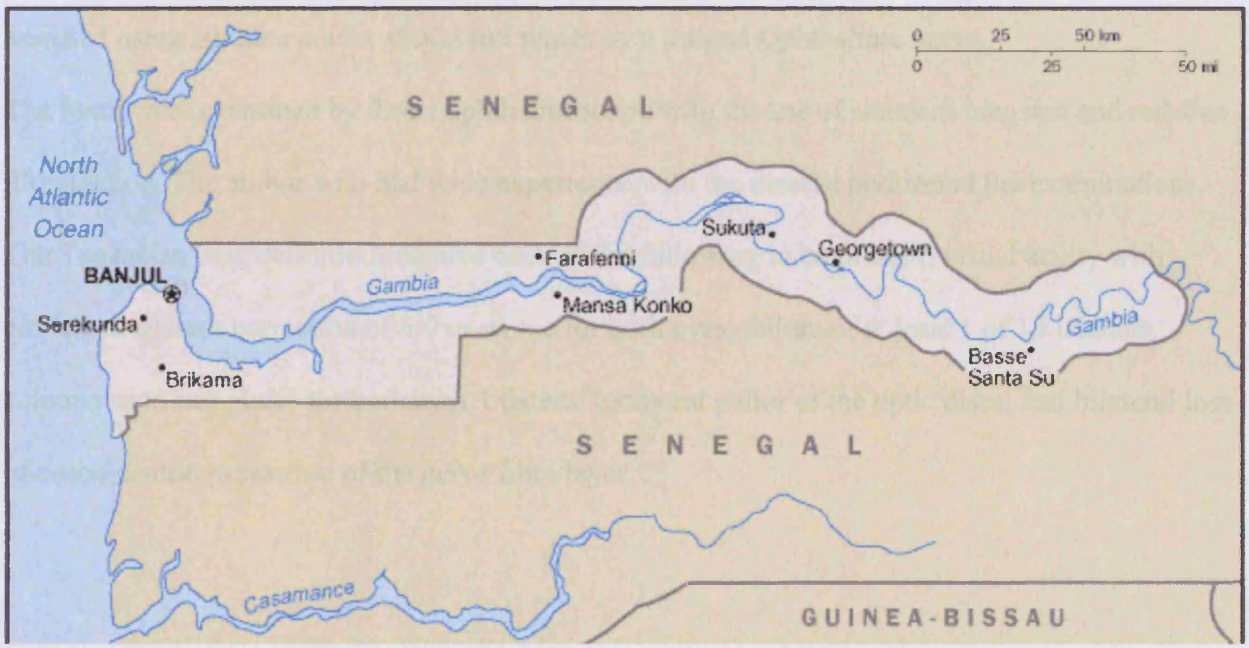
Tanzania is an Eastern African country, bordering the Indian Ocean, between Kenya and Mozambique. Its area is 945,000 sq km land: 886,000 sq km water: 59,000 sq km note: includes the islands of Mafia, Pemba, and Zanzibar. Population: 37 million, note: estimates for this country explicitly take into account the effects of excess mortality due to AIDS; this can result in lower life expectancy, higher infant mortality and death rates, lower population and growth rates, and changes in the distribution of population by age and sex than would otherwise be expected (July 2004 est.) Total fertility rate: 5.15 children born/woman (2004 est.)

Tanzania is one of the poorest countries in the world. The economy depends heavily on agriculture, which accounts for about half of GDP, provides 85% of exports, and employs 80% of the work force. Topography and climatic conditions, however, limit cultivated crops to only 4% of the land area. Tanzania adopted socialist political system from its independence, with the government subsidising basic commodities. In mid-eighties, the government has decided to adopt market-led economy, and stopped subsidising food; this has impacted on the poorest sections of society, and has precipitated the epidemic of optic neuropathy. Dar es Salaam, is the commercial capital with an estimated population of 3.8 million.

Fig 3.2: Somalia Map

Population: 11.5 million (July 2004). Its area is 627.000 sq km. Somalia's economic fortunes are being driven by its deep political divisions. Economic life continues, in part because much activity is local and relatively easily protected. Agriculture is the most important sector, with livestock normally accounting for about 40% of GDP and about 65% of export earnings. Nomads and semi-nomads, who are dependent upon livestock for their livelihood, make up a large portion of the population. Livestock, hides, fish, charcoal, and bananas are Somalia's principal exports, while sugar, sorghum, corn, qat, and machined goods are the principal imports. Somalia's small industrial sector, based on the processing of agricultural products, has largely been looted and sold as scrap metal. Despite the seeming anarchy, Somalia's service sector has managed to survive and grow. Telecommunication firms provide wireless services in most major cities and offer the lowest international call rates on the continent. Mogadishu is the capital city of Somalia, with an estimated population of 2.5 million (2003).

Fig 3.3: Gambian Map



Gambia is situated in West Africa, bordered by the Atlantic Ocean in the west,, and by Senegal in all other directions. Population: 1.4 million, land: 10.000 sq km (July 2004 est).

The Gambia has no important mineral or other natural resources and has a limited agricultural base. About 75% of the population depends on crops and livestock for its livelihood. Small-scale manufacturing activity features the processing of peanuts, fish, and hides.

Methods

3A- Prevalence studies:

3A1- Dar es Salaam Prevalence study:

We used stratified systematic random sampling to select a sample of 1078 persons, 10 to 39 years old, in the 21 residential areas of Dar es Salaam. The sample size was calculated based on study design, assumed prevalence rate and power to get a statistically significant confidence intervals.

The visual acuity was measured by an ophthalmic nurse, using snellen chart, and colour vision was assessed using Ishihara colour vision test plates by a trained Ophthalmic nurse.

The fundi were examined by direct ophthalmoscope with the use of standard tungsten and red-free illumination. The author who had wide experience with the disease performed the examinations.

The Tanzanian case definition requires each of the following to be present: visual acuity with pinhole or glasses correction of 6/9 or worse for both eyes, failure of at least 1 of 15 Ishihara colour-vision test plates for both eyes, bilateral temporal pallor of the optic discs, and bilateral loss of caeco-central projection of the nerve fibre layer.

A questionnaire was designed to collect the data on age, sex, residence, occupation, neurological symptoms, weight loss, breastfeeding (pregnant women were asked whether they were at the same time breastfeeding or not), from the sampled subjects. This data was entered into a computer database, and analyzed using SPASS software. In calculating estimates for prevalence and 95% confidence limits, the survey design was taken into account.

3A2- Gambian Study:

A multistage random sampling method was used. The country was stratified into seven health divisions. Within each region/division a sample of districts was randomly selected using proportional probability sampling (PPS) and, within each selected district, a sample of settlements was selected, again using PPS, with stratification by settlement size (small <400 residents and large 400+ residents). Within each settlement, a compound to compound census was undertaken to provide an up to date sampling frame from which a sample of compounds was selected. All residents within the selected compound were examined (Faal et al., 2000).

The field work was undertaken using three teams, each working in a different part of the country after standardization of examination methods. A two part ophthalmic examination was carried out. All subjects aged 5 years and older had measurement of visual acuity and an anterior segment examination by an ophthalmic medical assistant with a focused torch and ×2 loupe. Everybody aged 33 and over and any younger person with a visual acuity of less than 6/18 in either eye was referred for detailed examination by an ophthalmologist, who used a direct and an indirect ophthalmoscope and a hand held slit lamp in addition to the torch and loupe.

Every person aged 35 and over also had visual fields screened with a Henson CFA3000 visual field analyzer, intraocular pressure measured with a Schiotz tonometer, and the optic disc inspected. 33 cases of low vision or blindness in which optic neuropathy was a sole cause of vision loss were found during the survey. As part of the survey, we recorded each person's name, the name of the head of their household/compound and address. Within one month of the main survey, we were able to return to the villages and identify each of the 33 cases. Each case was invited to attend their nearest eye unit (Banjul, Bansang, Basse or Farafenni) on a specific day for clinical assessment. The clinical assessment involved the following:

Measurement of visual acuity (snellen chart) and colour vision (Ishihara plates), Standard ophthalmic examination (portable slit lamp & direct ophthalmoscope), Assessment of visual fields (Henson field analyzer) and colour fields (red target)

Fundus photography (Kowa fx-500 c fundus camera), Peripheral nerve assessment (knee and ankle jerk reflexes; temperature, pain, touch and joint position sensation; movement co-ordination), Hearing assessment (whisper and Rinne's test).

Cases were also asked their clinical history (ophthalmic and neurological questions) and lifestyle (use of medicines, diet, smoking, alcohol use and any weight loss at the time of visual loss).In calculating estimates for prevalence and 95% confidence limits, a total number of 6873 from the national sample, aged 10-40 were included, to get a proper comparison with the Tanzanian survey. The survey design (stratified cluster random sampling) was taken into account and any excess sampling error arising from the design (extrabinomial variation) were incorporated into the calculations.

3B- Case series studies:

3B1- Tanzanian case series study

15 Ophthalmic nurses from (Mnazi Moja, Kinondoni and Mtoni) which are three strategically located primary care clinics in Dar es Salaam were trained on case definition for a week. They were given visual acuity charts and Ishihara plates to screen for potential case patients and refer them to the dedicated study clinic. Every referred potential case was seen in the study clinic where they had an extensive ophthalmic and neurological examination to determine whether they were definite cases.

The case definition that has been employed in this study is as follows:

1- Bilateral, simultaneous visual loss, the interocular difference in acuity being no greater than two lines of the Snellen chart.
2- Impaired colour vision (Ishihara plates).
3- Bilateral central or caeco-central scotomas at onset.
4- Bilateral temporal pallor of the optic nerve, and/or hyperaemia of the optic disc.

All these criteria must be present for a subject to be included as a case in this study.

An ethical committee at the Ministry of Health, Tanzania, approved the protocol of the study.

Questionnaire:

Tanzanian Nurses trained in interviewing techniques administered a questionnaire regarding age, occupation, residence, place of work and exposure to any known toxic agents. The information collected included date of onset and how long it took for the visual loss to reach its worst level. The patients were asked in detail about past and present medication; their regular forms of food, and any weight loss in the last few months; drinks and smoking habits (Appendixes 1,2).

Ophthalmic examination:

A certified ophthalmic nurse determined best-corrected visual acuity, colour test with Ishihara pseuodo-isochromaqtic colour plates. Visual fields were charted by the author on a 1-m tangent screen with use of 1- to 10 mm red test objects. The author carried out slit-lamp examination and tonometry to exclude other causes of abnormal visual function. He then performed the funduscopic examinations. Fundus photographs were taken with a Kowa fx 50 R camera to confirm the results of the funduscopic examination.

Neurological examination:

The author obtained neurological histories and used bedside clinical methods to assess cranial nerves III to XII. Other neurological findings were based on assessment of tandem walking, heel-shin ataxia, vibration sensation at ankles, cotton wool sensation in lower limbs, knee and ankle jerk and joint position awareness.

Audiometric assessment:

A trained Tanzanian audiometry technician carried out all the audiometric measurements at the ENT department of Muhimbili Medical Centre. He used Bosch ST 10Audiometer, and carried out pure tone audiogram, and plotted his findings on an audiometric chart.

Statistical analysis:

The author entered the data into an excel data sheet and double checked it. Descriptive analysis was done using SPSSt for windows software. Simple, cross tabulations and multivariate analyses are used to show disease status ,exposure variables and to determine predictors of severe disease.

3B2- Somalian case series study

The case definition that has been employed in this study is as follows:

1- Bilateral, simultaneous visual loss, the interocular difference in acuity being no greater than two lines of the Snellen chart.

2- Impaired colour vision (Ishihara plates).

3- Bilateral temporal pallor of the optic nerve, and/or hyperaemia of the optic disc.

All these criteria must be present for a subject to be included as a case in this study.

An ethical committee at the Ministry of Health, Somalia, approved the protocol of the study. Nurses trained in interviewing techniques administered a questionnaire regarding age, sex, occupation, residence, place of work and exposure to any known toxic agents. The information collected included date of onset and how long it took for the visual loss to reach its worst level. The patients were asked in detail about past and present medication; their regular forms of food, and any weight loss in the last few months; drinks and smoking habits (Appendix 1).

Ophthalmic examination:

A certified ophthalmic nurse determined best-corrected visual acuity, colour test with Ishihara pseuodo-isochromaqtic colour plates. The author carried out slit-lamp examination and tonometry to exclude other causes of abnormal visual function. He then performed the funduscopic examinations.

The author entered the data into an excel data sheet and double checked it. Descriptive analysis was done using SPSSt for windows software. Simple tabulations are used to show disease status and the exposure variables.

3C- Aetiological studies:

3C1: Cyanide intoxication in Tanzanian ON cases and controls

31 cases within two weeks of disease onset with complete samples, and 14 controls were recruited to this study. The controls were attending the same primary eye care clinic because of disorders such as trauma or refractive error without any clinical evidence of optic neuropathy. The controls had the same age and sex distribution and belonged to the same socio-economic group as the cases. All cases and controls were non-smokers. Urine and blood samples were taken from the cases and controls and stored frozen at – 20 degrees centigrade until analysis. We have carried out laboratory tests on urine, and serum samples at the laboratory of the Tanzanian Food and Nutrition Centre in Dar es Salaam. We used the method described by Lundquist et al. (1979) to determine thiocyanate in serum and urine. This method is used for rapid and specific measurement of thiocyanate in serum or urine. It involves separating thiocyanate from interfering compounds by adsorbing it on an anion-exchange resin that has special affinity for thiocyanate, then eluting with sodium perchlorate. The eluted thiocyanate is quantified by a modified Konig reaction, sodium hypchlorite being used as the chlorinating agent.

3C2: Leber's hereditary optic neuropathy as a cause for this epidemic:

Whole blood EDTA samples were taken from the 31 cases and 14 controls. DNA was extracted from leucocytes and was then screened for the three primary mutations most commonly associated with LHON using standard assays. These assays were done at the neurogenetics laboratory of the Institute of Neurology , London, with thanks to Professor Nick Wood.

3C3: Vitamin status of ON cases and control subjects

Different blood samples were obtained from 14 subjects who were clinically free of symptoms (controls), and 16 subjects who were suffering from optic neuropathy (cases). Blood samples obtained from venopuncture were kept frozen until analyzed in Dunn Nutrition centre in Cambridge.

Riboflavin levels were measured by the ratio of the activity of glutathione r Riboflavin levels were measured by the ratio of the activity of glutathione reductase with added FAD cofactor to the activity without added FAD, *in vitro* (EGRAC = Erythrocyte Glutathione Reductase Activation Coefficient). Serum folate was measured by the Abbott IMx ion capture procedure. Vitamin B12 concentrations were measured by the Abbott IMx ion capture method. Erythrocyte aspartate aminotransferase is a measure of vitamin B_6status, it increase in deficiency. Zinc levels are measured by Superoxide dismutase activity. This enzyme's activity would fall with severe zinc or copper deficiency.

3D: Treatment study

225 acute cases of Tanzanian epidemic optic neuropathy were recruited to the treatment study. They were given vitamin B and multi-mineral supplements tablets (Sanatogen) to be taken daily for 3 months (one tablet /everyday).

There were no control subjects for this study, as the Tanzanian authority granted the ethical approval of this study conditional to all cases receiving vitamin supplements. Ophthalmic, neurological and audiometric examinations were undertaken at baseline and follow-up visits (1 month and 3 months).

Table 3.2: vitamin and mineral contents of Sanatogen tablets (Roche)

Typical values	% RDA	Per tablet
Vitamin A	100	800μg
Vitamin D	100	5μg
Vitamin E	100	10 mg
Vitamin C	100	60 mg
Thiamine	100	1.4 mg
Riboflavin	100	1.6 mg
Niacin	100	18 mg
Vitamin B6	100	2 mg
Folic Acid (Folacin)	100	200 μg
Vitamin B12	100	1 μg
Biotin	100	0.15 mg
Pantothenic Acid	100	6 mg
Beta Carotene		400 μg
Lutein		275 μg
Vitamin K		30 μg
calcium	22	173 mg
Phosphorus	18	144 mg
Iron	100	14 mg
Magnesium	40	120 mg
Zinc	100	15 μg
Iodine	100	150 μg
Copper		2 mg
Boron		150 μg
Chloride		36.3 mg
Chromium		25 μg
Manganese		2.5 mg
Molybdenum		25 μg
Nickel		5 μg
Potassium		40 mg
Selenium		25 μg
Silicon		2 mg
Tin		10 μg
Vanadium		10 μg

Chapter 4

Results

4A- Prevalence Studies

4A1: Tanzanian community survey

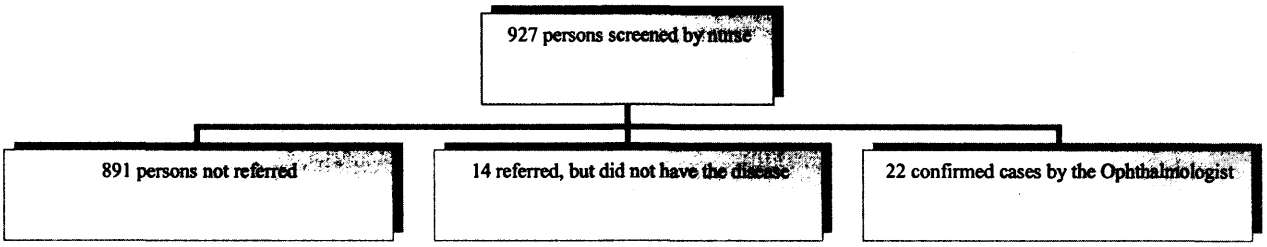
A sample of 1078 were selected as a sample, of whom 927 (86 percent) were examined for signs of the disease. 57% of the examined subjects were females, while 43% were males Mean age of subjects was 22.1 years (standard deviation 7.5 years), for males it was 22.3 years (SD: 2.9 yrs), and for females it was 22.1 years (SD: 2.1yrs) . Twenty-two cases were identified (prevalence, 2.4%; 95 percent confidence interval, 1.7 to 3.0 percent). 8 cases were found among the 392 males examined (prevalence, 2.1 per cent), and 14 among the 535 females examined (prevalence, 2.7 percent). The female age group 20 – 29 had the highest prevalence of 4.3 %, as for the males it was the age group 10 – 19 with the highest prevalence at 2.6%.

Table 4.1: Age/Sex comparison between persons seen, and sampled persons not seen

Age years	Males %		Females %		Total %	
	Seen	Not seen	Seen	Not seen	Seen	Not seen
10 -19	44	43	56	57	41	39
20 – 29	37	39	61	61	40	37
30 - 39	49	48	51	52	21	24

There was no significant difference in age/sex characteristics between those seen by team, and those sampled person but not traced by the team.

Table 4.2:Flow-chart of persons screened by the nurse, and confirmed cases



Of 927 screened by the nurse,36 were referred to the ophthalmologist, who confirmed that 22 cases had the optic neuropathy.

Table 4.3: Prevalence of Optic neuropathy in Dar, Tanzania by Age/Sex

Age in	Male			Female			Total		
years	Normal	Cases	%	Normal	cases	%	Normal	cases	%
10 – 19	156	4	2.3	195	2	1.02	351	6	1.9
20 – 29	137	2	1.5	230	10	4.3	367	12	3.0
30 – 39	91	2	2.2	96	2	2.1	187	4	2.2
Total	384	8	2.1	521	14	2.7	905	22	2.4

Table 4.4: Visual Acuity of 22 Tanzanian prevalent Optic neuropathy cases

Best corrected visual acuity	Number	%
6/9 – 6/18	16	73
6/24 – 6/60	6	17
Total	22	100

Over 70% of the cases had moderate loss of visual acuity, while 17% had severe visual loss at the time of examination. These cases were found in the community, and it is striking that they did not seek medical attention with this level of visual acuity loss.

Table 4.5: Colour vision of Tanzanian prevalent optic neuropathy cases

No of Ishihara plates correctly read out of 15	Number	%
10 – 14	10	45.4
5 – 9	6	27.3
1 – 4	6	27.3
Total	22	100.0

45.4% of cases had mild loss of colour vision, while the remaining 54.6 of cases presented with moderate to severe loss of colour vision.

Table 4.6: Symptoms of peripheral neuropathy in Tanzanian prevalent cases

Symptoms of peripheral neuropathy	Number	%
Numbness in legs	8	36.4
Burning sensation in legs	8	36.4
No peripheral symptoms	6	27.2
Total	22	100.0

36% of cases complained of peripheral neuropathy symptoms, mainly numbness and burning sensation at the legs.

Table 4.7: Hearing symptoms of Tanzanian prevalent cases

Hearing loss (self reported)	Number	%
Yes	7	31.8
No	13	68.2
Total	22	100.0

About 32% of cases reported having hearing loss coinciding with the onset of visual symptoms.

Table 4.8: Other features of Tanzanian prevalent ON cases

Characteristic	Number	%
Stomatitis	12	54.5
Weight loss (self reported)	5	22.7
Duration of disease(months)		
< 6	11	50.0
6 – 11	5	22.7
≥12	6	27.3
Total	22	100.0

Stomatitis was found in 54% of cases, this is indicative of vitamin B deficiency in our cases. Weight loss in the 2 months preceding the disease onset was reported by 22% of cases. The duration of the disease indicates that it has been affecting the community for many years, but has increased in prevalence for the last year, as 72.3% of our cases reported noticing the visual symptoms within one year of being examined.

Table 4.9: Association of Breastfeeding and Tanzanian optic neuropathy

Breastfeeding	Normal	Cases	%
Yes	28	7	20.0
No	369	6	1.6
Not applicable	124	1	0.8
Total	521	14	2.6

Breastfeeding was a strong risk factor. Seven cases were found among the 35 women who were breast-feeding (20.0 percent), as compared with six cases among the 375 women of reproductive age who were not breast-feeding (1.6 percent; prevalence ratio, 12.5; 95 percent confidence interval 4.4 to 35.2). It is worth noting that pregnant women were asked whether they were breastfeeding at the same time or not, and were included accordingly.

4A2 Gambian Prevalence Study:

A high response rate was achieved: 90.9% (30/33) were traced and examined. The population of the Gambia was estimated to be 1.17 million in 1996. The population is currently growing by 4% per year, and life expectancy had increased from 43.5 in 1983 to 53.5 in 1993. 4 cases of epidemic optic neuropathy were found, among 6873 sampled persons aged 10-40. This means a prevalence of 0.06%, with 95% confidence interval of 0.04 – 0.08. The mean age of epidemic optic neuropathy was 36 years (standard deviation: 6.2 years). For males it was 33 years, and for females it was 37 years (standard deviation: 7.21). Females represented 75% of epidemic optic neuropathy cases in the Gambia.

Table 4.10: Coverage of Gambian optic neuropathy cases

Status	N	%
Traced & Examined	30	90.0
Migrated	2	6.1
Not traced	1	3.0
Total	33	100.0

Table 4.11: Clinical Diagnosis of Gambian optic neuropathy cases

Diagnosis	N	%
Bilateral Optic Atrophy	6	20.0
Unilateral Optic Atrophy	9	30.0
Chorioretinal Scar	6	20.0
Congenital Optic disc hypoplasia + maculopathy	4	13.3
Other ocular pathology	5	16.7
Total Examined	30	100.0

Bilateral optic atrophy represented only 20% of all the optic neuropathy cases.

Table 4.12: Etiology of Gambian Bilateral Optic Atrophy

Diagnosis	N	%
Epidemic Optic Neuropathy	4	66.6
Macular Fibrosis	1	16.7
Unknown origin	1	16.7
Total	6	100.0

Epidemic Optic Neuropathy was seen in 4 cases out of 6873 subjects aged 10 – 40 years examined during the Gambia survey. This results in epidemic optic neuropathy prevalence of 0.06% in the Gambia.

Table 4.13: Visual Acuity of Epidemic Optic neuropathy cases in Gambia

Visual Acuity	N	%
6/9 – 6/18	1	25
6/24 – 6/60	3	75
Total	4	100

75% of epidemic optic neuropathy cases had moderate to severe visual loss at the time of the survey. These are prevalent cases diagnosed in the community.

Table 4.14: Colour Vision of Epidemic Optic neuropathy cases in Gambia

No of Ishihara plates correctly read out of 15	N	%
10 – 14	1	25
5 - 9	1	25
1- 4	2	50
Total	4	100

50% of cases were not able to read more than 4 plates of Ishihara plate at the time of diagnosis.

Table 4.15: Symptoms of peripheral neuropathy in the Gambian cases

Symptoms of peripheral neuropathy	N	%
Numbness in legs	3	75
Burning sensation in legs	3	75
No symptoms	1	25
Total	4	100

75% of optic neuropathy cases complained of peripheral neuropathy symptoms, mainly numbness and burning sensation in the legs.

Table 4.16: Hearing symptoms of the Gambian cases

Hearing loss (self reported)	N	%
Yes	1	25
No	3	75
Total	4	100

25% of cases presented with hearing loss at the time of disease onset.

Table 4.17 Other features of Gambian cases

Characteristic	N	%
Weight loss	2	50
Duration of disease (yrs)		
< 1 yr	1	25
1-2 yrs	1	25
> 2yrs	2	50
Breastfeeding		
Yes	2	50
No	1	25
Not applicable	1	25
Total	4	100

50% of cases reported weight loss at the time of disease onset. The disease is endemic in the Gambia as about 50% of cases had it, for more than 2 years, and only 25% of the cases reported noticing the visual loss within one year of being examined.

4B: Case Series Studies:

4B1Tanzanian case series study:

Table 4.18: Age/ Sex distribution of Tanzanian optic neuropathy cases

Age group in years	Males		Females		Total	
	No	%	No	%	No	%
10 - 19	46	38.4	27	25.7	073	32.4
20 - 29	49	40.8	55	52.4	104	46.2
30 - 39	25	20.8	23	21.9	048	21.4
Total	120	100.0	105	100.0	225	100.0

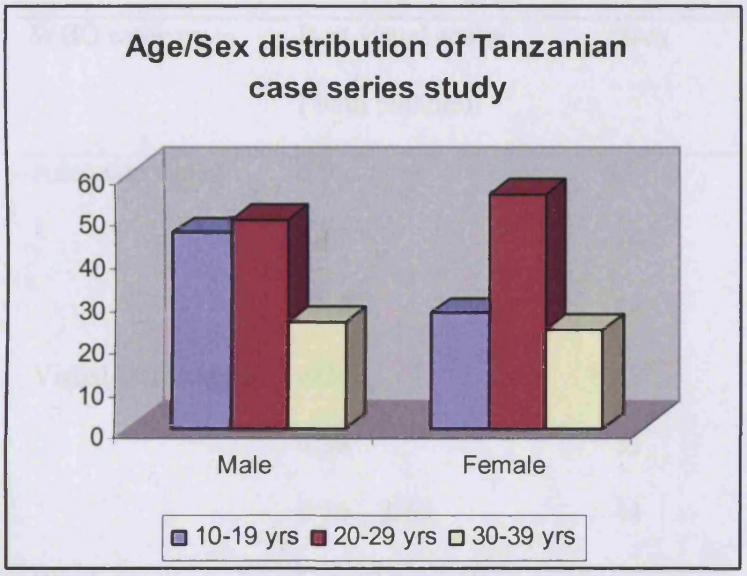


Fig 4.1: Histogram showing Age/Sex distribution of Tanzanian cases

225 patients matched the selection criteria and were included in the study.

Age/Sex distributions of patients are detailed in Table 1. Males represented 53.3% of patients, while females were 46.7%. The mean age of the patients was 25.5 years (standard deviation was 6.9 years). Mean age of males was 23.2 (standard deviation was 6.8 years). Mean age of females was 23.8 years (standard deviation was 6.3 years).

Table 4.19: Time interval between onset of vision loss and attending Dar clinic

Interval	Cases	%
1 - 2 weeks	031	013.8
3 - 4 weeks	088	039.1
5 - 8 weeks	072	032.0
9 - 12 weeks	034	015.1
Total	225	100.0

More than half of our patients attended the clinic, within one month of onset of vision, while about 14 % of cases presented within two weeks of disease onset. This is the first time a study on acute cases of optic neuropathy has ever been taken.

Table 4.20: Best corrected visual acuity of Tanzanian ON cases at presentation

WHO category	Best visual acuity (with pinhole)	cases	%
Adequate vision	6/9	12	5.3)
	6/12	14	6.2)
	6/18	28	12.4) 23.9
Visual Impairment	6/24	42	18.6)
	6/36	83	36.9)
	6/60 – 3/60	44	19.7) 75.2
Blind	Cannot see 3/60	2	0.9) 0.9
Total		225	100.0

76% of cases had best corrected visual acuities less than 6/18, which is visual impairment category of WHO, 2 patients were categorized as blind, as their best visual acuity was less than 3/60.

Table 4.21: VA at presentation in relation to duration of disease at presentation

Duration in weeks	VA at presentation > 6/18	6/24-3/60	<3/60	Total
< 2	16	15		31
3 – 4	14	74		88
5 – 8	14	57	1	72
9 - 12	10	23	1	34
Total	54	169	2	225

There was no clear pattern of disease severity, in relation to duration of disease at presentation.

Table 4.22: Best colour vision of Tanzanian cases based on 14 Ishihara test plates

No of plates seen	Cases	%
14	0	0.0
10 - 13	76	33.8
5 - 9	87	38.6
1 - 4	60	26.7
Unable to read control plate	2	0.9
Total	225	100.0

At presentation, 65% of cases were failing between 5-14 Ishihara plates, and the two blind cases were not able to see the Ishihara control plates.

Table 4.23: Time from onset to visual loss of Tanzanian cases

Time of Visual loss	Cases	%
< 2 weeks	115	51.1
2 – 4 weeks	35	15.6
5 – 8 weeks	68	30.2
> 8 weeks	7	3.1
Total	225	100.0

51.1% of cases lost their vision within two weeks of diseases onset, while 97% of all patients had their vision deteriorated within 2 months of noticing visual symptoms.

Table 4.24: Ophthalmic Assessment of Tanzanian cases at presentation

Ophthalmic assessment	Cases	%
Pupillary response		
Normal	165	73.3
Slow	054	24.0
Relative Afferent Pupillary Defect (RAPD)	006	02.7
Colour field/Amsler charts		
Normal	030	13.4
Abnormal	195	86.6
Central scotoma	152	67.5
Centro-caecal scotoma	039	17.3
Other scotoma	003	01.3
Not assessed	001	0.4
Optic Disc (OD)		
Normal	0	0
Abnormal	225	100
Blurred OD margins	045	020.0
Dilated OD capillaries	093	041.3
Temporal OD pallor	151	067.1
Temporal pigmentation	091	040.4
Atrophy	007	003.1
Other	005	002.2
Nerve fibre layer		
Normal	1	0.4
Abnormal	224	99.6
Loss of papillo-macular bundle	155	68.9
Thickening of nerve fibre layer	180	80.0
Loss of foveal reflex	056	24.9
Other	008	3.6
Total	225	100.0

Table 4.25: Audiometric Assessment of Tanzanian cases at presentation

Sensori-neural hearing		Case	%
Normal	< 20 dB loss	64	34.0
Mild impairment	20 - 30 db loss	83	44.1
Moderate impairment	40 - 59 dB loss	40	21.3
Severe impairment	60+ dB loss	1	0.5
Total tested		188	100

66% of cases had sensori-neural hearing loss at presentation.

Table 4.26: Neurological symptoms reported by Tanzanian cases at presentation

symptom	Cases	%
Sensory symptoms in lower limbs	119	52.9
Burning sensation in feet	72	32.0
Numbness in legs	112	49.8
Constant sensory symptoms in lower limbs	40	17.8
Burning sensation in feet	21	9.3
Numbness in legs	38	16.9
Other symptoms	70	31.1
Difficulty walking	21	9.3
Difficulty controlling bladder	22	9.7
Difficulty with memory	35	15.6
Total cases interviewed	225	100

These were self-reported symptoms at the time of visual loss onset.

Table 4.27: Neurological Examination of Tanzanian cases

Neurological examination	Cases	%
Signs of peripheral neuropathy		
No signs	145	65
Signs of peripheral neuropathy	079	35
Total examined	224	100.0

Based on assessment of tandem walking, heel-shin ataxia, vibration sensation at ankles, cotton wool sensation in lower limbs, knee and ankle jerk and joint position awareness. About 35% of patients had signs of peripheral neuropathy at presentation, while 65% of patients were optic neuropathy cases.

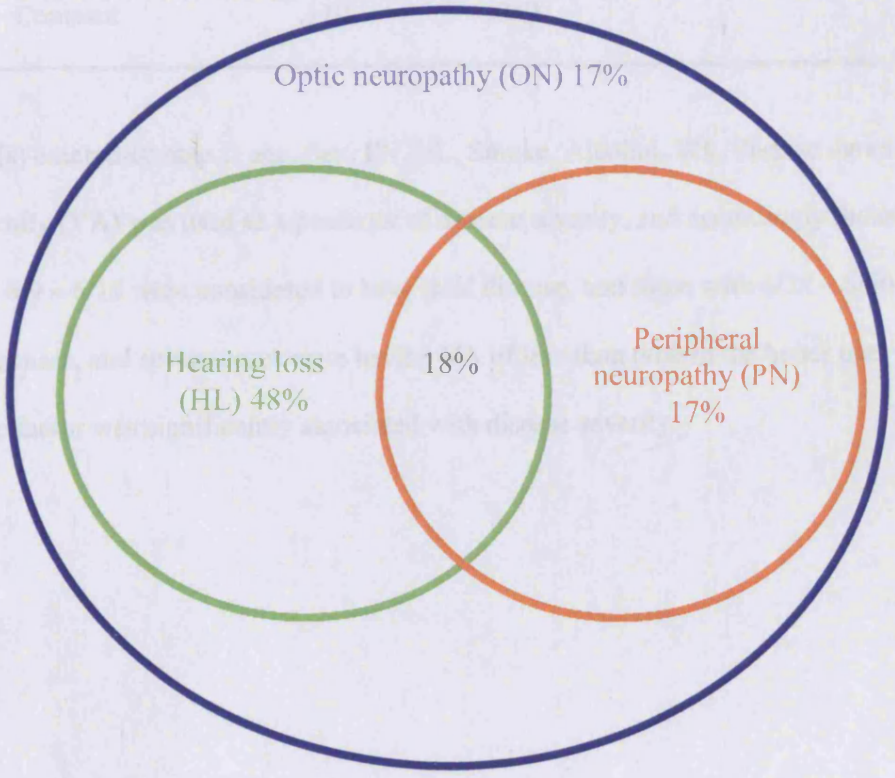


Fig 4.2: Association of Optic Neuropathy, hearing loss and peripheral neuropathy in Tanzanian cases.

Table 4.28 Multivariate Analysis of Tanzanian case series

		p-value	Odds ratio	95.0% C.I. for odds ratio	
				Lower	Upper
Step 1(a)	age	.089	2.420	1.900	6.155
	Sex	.079	2.217	.912	5.386
	PN	.110	.867	.727	1.033
	HL	.281	.906	.758	1.084
	Smoke	.316	2.112	.490	9.106
	Alcohol	.159	.489	.181	1.323
	WL	.565	1.351	.484	3.768
	Disease duration	.074	.616	.362	1.048
	Constant	.110	.030		

Variable(s) entered on step 1: age, Sex, PN, HL, Smoke, Alcohol, WL, disease duration.

Visual acuity (VA) was used as a predictor of disease severity, and accordingly those with VA 6/9 – 6/18 were considered to have mild disease, and those with 6/24 – 6/60 as moderate cases, and severe cases were having VA of less than 6/60 in the better eye.

No single factor was significantly associated with disease severity.

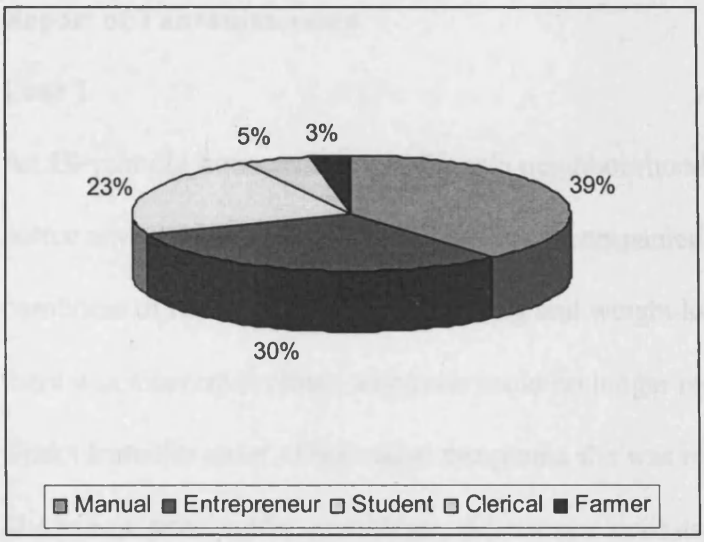


Fig 4.3: Occupation of Tanzanian cases

39% of cases were manual labourers, while further 30% were entrepreneurs. This shows that majority of the cases were from low socio-economic level.

Report of Tanzanian cases

Case 1

An 18-year old housewife from Mbagala neighbourhood of Dar es Salaam, began to notice a worsening of her vision. This was accompanied by burning sensation and numbness of her feet, difficulty in hearing and weight loss. She recognized that there was a severe problem when she could no longer recognize faces. After 2 weeks from the onset of her visual symptoms she was referred to our study clinic. She had no prior medical problems, did not smoke or drink alcohol, but was breastfeeding for the last 6 months. On examination, best corrected bilateral visual acuity was 6/60, colour vision she could discern 3 of 14 Ishihara plates. On colour field testing, she demonstrated a caeoco-central scotoma in both eyes. Fundus examination demonstrated fairly normal fundus, apart from swollen and hyperaemic discs. The findings of her neurological examination were normal. She had mild sensori-neural hearing loss. She was started on vitamin supplements for three months, and came back for follow-up assessments at 1 month and 3 months from presentation.

At the one month follow-up assessment, her visual acuity improved to 6/12. Optic nerve function studies were also improved: She could discern 6 of 14 Ishihara plates, colour field tests were entirely normal. Fundus examination showed a thinning of the nerve fibre layer in the papillomacular bundle in both eyes and marked decrease of the optic disc swelling. At 3 month assessment, visual acuity was 6/4, colour vision 14 out of 14 Ishihara plates. Her hearing returned to normal, and the neurological symptoms disappeared. The fundus examination showed loss of papillomacular bundle and temporal pallor of the optic discs.

Case 2

A 30 year-old man living in Ubongo area of Dar es Salaam and working as a car mechanic, was well until he noticed sudden visual symptoms, which began as a clouding of central vision in the left eye and progressed in a few days to the right eye. In a week, he could no longer work or watch television. He had no other medical history. He did not smoke nor drink alcohol, and did not notice any weight loss coinciding with the visual symptoms.

He came to our clinic within one week of onset of his symptoms. On our examination, best bilateral visual acuity was 6/60. He could recognize 3 of fourteen Ishihara plates.

Pupil examination showed both pupils to have a sluggish response to light, but there was no afferent papillary defect. Colour field testing showed bilateral dense central scotomas. A fundus examination demonstrated a normal appearing nerve fibre layer, and swollen and hyperaemic optic discs.

Neurological examination was normal, and there was a mild sensori-neural hearing loss on audiometry testing. He was started on vitamin supplements, and told to come back for 1 month and 3 months follow-up assessments.

On re-examination after one month of vitamin supplements, the visual acuity improved and was 6/9, while he could see all the 14 Ishihara plates. On fundus examination there was temporal pallor and loss of the nerve fibre layer in the papillomacular bundle.

Again at 3 month, the visual acuity further improved, and at this time his bilateral visual acuity was 6/4, and colour vision was 14/14, but the temporal pallor and nerve fibre layer loss at the papillomacular bundle remained.

Case 3

A 15 year old student from Mtoni area of Dar es Salaam, was well, until she noticed that she could no longer read or recognize faces. She also noted that she could not see any colours and believed that her hearing was impaired bilaterally on an intermittent basis. She came to the clinic after 3 months from the start of her symptoms.

On examination, her bilateral visual acuity was 6/60. She could discern only 3 out of 14 Ishihara plates, and there was caeco-central scotoma on testing her colour field. Fundus examination demonstrated severe temporal pallor of both optic discs and severe losses of the nerve fibre layer in the papilomaculare bundle.

General neurological examination revealed signs of peripheral neuropathy, mainly of decreased sensation to pinpricks and cotton wool in her legs.

After one month of vitamin supplements, the vision improved to 6/36, and colour vision was 6/14 Ishihara plates. At three months assessment the vision was 6/9, and colour vision was 14/14.The central scotoma, and the peripheral neurpathy have disappeared. The fundus picture remained the same, with temporal pallor of the optic discs, and nerve fibre layer loss at the papillomacular bundle.

4B2- Somalian case series study:

Table 4.29: Age/Sex distribution of Somalian ON cases at presentation

Age in years	Male		Female		Total	
	No	%	No	%	No	%
10-19	19	27.9	10	27.1	29	27.6
20-29	34	50.0	16	43.2	50	47.6
30-39	15	22.1	11	29.7	26	24.8
Total	68	64.8	37	35.2	105	100.0

65% of cases were male, compared to 35% female. The mean age of the cases was 24 years (standard deviation 5.3 years).

Table 4.30: Visual Acuity of Somalian ON cases at presentation

Visual Acuity	No	%
6/9 – 6/18	20	19.0
6/24 – 6/60	75	71.5
5/60 - 3/60	10	9.5
Total	105	100.0

81% of the cases had visual impairment at the time of their presentation to the clinic.

Table 4.31: Colour Vision of Somalian ON cases at presentation

Plates seen based on14 Ishihara plates	No	%
10-14 plates	10	10
5 – 9 plates	18	17
1- 4	77	73
Total	105	100

Over 70% of the cases had deteriorated colour vision, as they were able to see only 1-4 test plates.

Table 4.32: Duration of disease of Somalian ON cases at presentation

Duration	No	%
< 1 month	31	30
2 – 3 months	37	35
4 – 6 months	22	21
7- 12 months	13	12
> 12 months	02	2
Total	105	100

30% of the cases noticed the visual symptoms for less than a month, prior to their presentation to the clinic.

Table 4.33: Other symptoms of Somalian ON cases at presentation

Symptom	cases	
	No	%
Hearing loss	30	28.5
Numbness at legs	62	59.0
Weight loss	47	44.7
Smoking	10	9.5
Stomatitis	28	26.6
Breastfeeding	16	51.6%

28.5% of cases had hearing loss at presentation and 59% of cases reported having numbness at legs which coincided with the visual loss. 44.7% of the Somalian cases noticed weight loss, and 51.6% of females at breastfeeding age were breastfeeding when they were affected by the optic neuropathy.

Table 4.34: Age/Sex distribution of Somalian (SO) and Tanzanian (TZ) ON cases at presentation

Age group in years	Males %		Females %		Total %	
	SO	TZ	SO	TZ	SO	TZ
10 - 19	28	38	27	26	28	32
20 - 29	50	41	43	52	47	46
30 - 39	22	21	30	22	25	22
Total	100	100	100	100	100	100

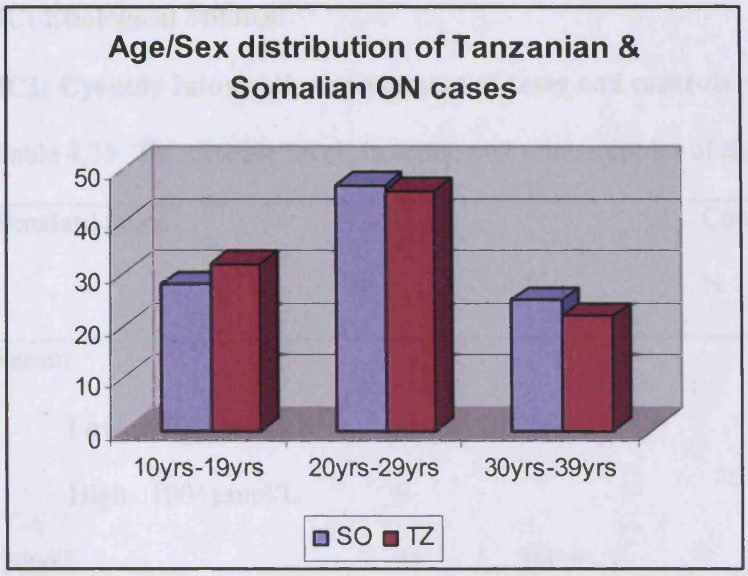


Fig 4.4: Age/Sex distribution of Tanzanian and Somalian cases was very similar, this supports our hypothesis that the condition is the same in the two locations.

4C: Etiological Studies:

4C1: Cyanide Intoxication assessment of cases and controls

Table 4.35: Thiocyanate levels in serum and urine samples of cases and controls

Standard range	Cases		Controls	
	N	%	N	%
Serum				
Low <100μmol/L	31	100.0	14	100.0
High 100+μmol/L	0		0	
Total	31	100.0	14	100.0
Mean (sd)	27.34μmol/L (25.7)		22.95μmol/L (24.5)	
Urine				
Low <100μumol/L	31	96.9	4	26.7
High 100+μmol/L	1	3.1	11	73.3
Total	32	100.0	15	100.0
Mean	36.26μmol/L (29.4)		48.42μmol/L (36.8)	

There is no significant difference of thyiocyanate levels between cases and controls. (p-Value of 0.31 for serum, and p value of 0.32 for urine analysis).

4C2- Leber's hereditary optic neuropathy as a cause for the epidemic

All DNA samples were negative for the 11778, 3460 and 14484 mutations which are found in 95% of Caucasian families with LHON.

4C3- Vitamin Status of cases and controls:

Thiamine analysis on the Tanzanian cases and controls

The transketolaseassay of thiamine status yields a ratio of stimulated (+ cofactor) to basal (- cofactor) activities, known as ETKAC, which goes up in deficiency. A second related index of thiamine status is the basal TK activity, given as "Activity values" with a haemoglobin denominator: (this is in IU/g haemoglobin). This index goes down in deficiency.

Table 4.36: Erythrocyte transketolase assay (ETKAC)

Standard range	ratio	Cases N	%	Controls N	%
Normal	1.00-1.15	6	37.6	6	40.0
Marginal	1.16-1.25	5	31.2	2	13.3
Deficient	>1.25	5	31.2	7	46.7
Total		16	100.0	15	100.0
Mean (sd)		1.20 (0.11)		1.20 (0.12)	

Table 4.37: Mean values and significant tests of Thiamine status

Index	Mean for Tanzanian cases			Mean for Tanzanian controls			t-test cases vs. controls	t-test Tanzania vs. UK
	Mean	SD	(n)	Mean	SD	(n)		
Thiamine : (transketolase)								
ETKAC	1.20	(011)	(16)	1.20	(0.12)	(15)	Not significant	t=3.53
ETK (basal) IU/g HB	0.56	(0.17)	(16)	0.56	(0.11)	(15)	Not significant	t=6.82 significant

There was no difference between cases and controls of the Tanzanian set, but the mean ETKAC was significantly higher than UK elderly. In the case of ETK (basal), again there is no difference between the cases and control, but a highly significant difference when compared with the UK elderly dataset (NDNS). Therefore the Tanzanian population is generally more deficient in Thiamine than those of the UK.

Table 4.38: Serum folate levels of the Tanzanian cases and controls

Standard range	Cases		Controls		t-test cases vs.
	N	%	N	%	controls
Normal 14.0+nmol/L	8	57.1	11	73.3	
Marginal 7.0-13.9 nmol/L	6	42.9	4	26.7	
Deficient <7.0 nmol.L	0	0.0	0	0.0	
Total	14	100.0	15	100.0	
Mean (sd)		16.7 (5.4		19.2 (8.8)	Not significant

None of the samples fell below 7nmol/L, which is the threshold of folate deficiency.

Table 4.39: Riboflavin (B2) status of the Tanzanian cases and controls

Standard range	Ratio	Cases		Controls		t-test cases vs. controls
		N	%	N	%	
Normal	1.00-1.29	1	0	0	0	
Marginal	1.30-1.79	1		2	14.3	
		6.2				
Deficient	1.80+	15		12	85.7	
		93.8				
Total		16		14	100.0	
		100.0				
Mean (sd)		2.98 (0.67)		2.86 (1.07)		Not significant

Almost all cases and controls were deficient in riboflavin.

Table 4.40: Vitamin B12 status of the Tanzanian samples

Standard range	Ratio	Cases		Controls		t-test cases vs. controls
		N	%	N	%	
Normal	>223pg/ml	7	50.0	11	78.7	
Intermediate	179-223 pg/ml	2	14.2	2	14.2	
Deficient	≤179pg/ml	5	35.7	1	7.1	
Total		14	100.0	14	100.0	
Mean (sd)		474 (287)		330 (245)		Not significant

Both cases and control had normal Vitamin B_{12} levels

Table 4.41: Vitamin B_6 status of the Tanzanian samples

Standard range	Ratio	Cases		Controls		t-test cases vs. controls
		N	%	N	%	
Normal	1.6 – 2.00nmol/L	9	56.3	13	86.7	
Marginal	2.0 – 2.5 nmol/L	5	31.2	2	13.3	
Deficient	>2.5 nmol	2	12.5	0	0	
Total		16	100.0	15	100	
Mean (sd)		2.06 (0.32)		1.87 (0.22)		T=1.91 (border line)

These Tanzanian results are within the normal range, but are a little higher in cases than the controls.

Table 4.42: Zinc levels of the Tanzanian samples

Standard range	ratio	Cases		Controls		t-test cases vs. controls
		N	%	N	%	
Normal	1092 – 1817 U/gHb	14	87.5	15	100	
marginal	1090 – 850 U/gHb	2	14.5	0	0	
Deficient	< 850 U/gHb	0	0	0	0	
Total		16	100	15	100	
Mean (sd)		1319 (252)		1302 (182		Not significant

Most Tanzanian results are within the normal range, and there is no difference between cases and controls.

Table 4.43: fat-soluble vitamin analysis of the Tanzanian samples

The fat soluble vitamins are analyzed directly from plasma samples.

	Tanzania controls			Tanzania cases			UK National Diet and Nutrition Survey (NDNS) (65-74 y) Finch S et al (1998)		
	n	Mean	(sd)	n	Mean	(sd)	n	mean	(sd)
Retinol	15	0.912	(0.441)	14	0.891	(0.343)	1079	2.148	(0.648)
Alpha-tocopherol	15	17.5	(4.8)	14	19.1	(3.8)	1079	35.7	(11.2)
Gamma-tocopherol	15	0.66	(0.30)	14	0.68	(0.35)	1071	2.29	(1.10)
Retinol palmitate	15	0.108	(0.106)	14	0.075	(0.031)	1018	0.078	(0.044)
Lutein	15	0.453	(0.277)	14	0.646	(0.449)	1079	0.363	(0.188)
Alpha-cryptoxanthin	15	0.020	(0.020)	14	0.020	(0.009)	1072	0.034	(0.036)
Beta-cryptoxanthin	15	0.075	(0.088)	14	0.071	(0.052)	1061	0.136	(0.143)
Lycopene	15	0.339	(0.171)	14	0.392	(0.202)	1072	0.249	(0.191)
Alpha-carotene	15	0.130	(0.253)	14	0.019	(0.007)	1068	0.070	(0.067)
Beta-carotene	15	0.334	(0.510)	14	0.130	(0.084)	1078	0.353	(0.236)

There was no significant difference between the Tanzanian cases and controls. There is clear deficiency in retinol and tocopherol levels of the Tanzanian samples compared to the British samples in the NDNS study.

4D- Treatment study:

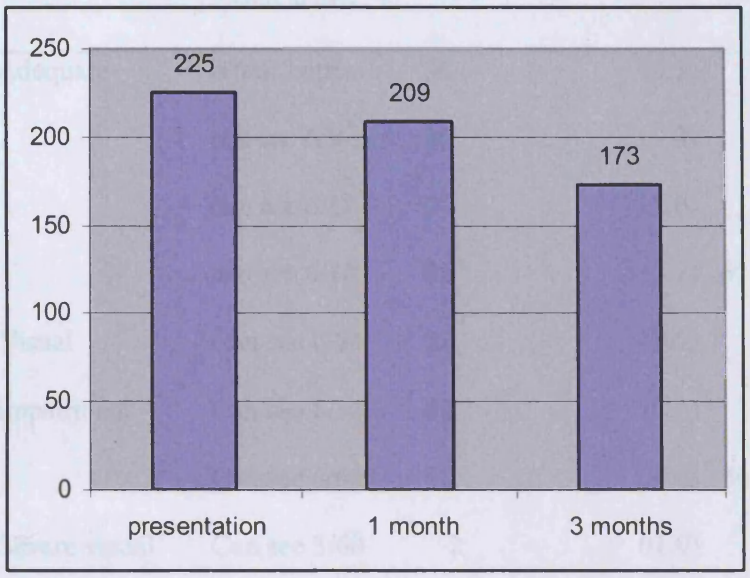


Fig 4.5: Follow-up of cases with supplements

This chart shows that 100 % of cases were seen at presentation, while 93% were seen at one month follow-up, and a further 77% of cases completed the 3-month vitamin supplements and came back to the clinic for assessment. Among those who were seen at 3 months, there were 14 (6%) who did not come back for their 1 month assessment. This results in only 2 cases (1%) that did not come back after their baseline assessment.

Table 4.44: Best corrected visual acuity outcome after 1 month of vitamin supplements

WHO	Best corrected visual acuity	Cases	%	
Adequate	6/6 or better	34	16.3)	
	can see 6/9	36	17.2)	
	can see 6/12	27	13.0)	
	can see 6/18	32	15.2)	61.7
Visual	Can see 6/24	27	13.0)	
impairment	Can see 6/36	32	15.2)	
	Can see 6/60	18	08.6)	36.8
Severe visual impairment	Can see 3/60	2	01.0)	
Blind	Cannot see 3/60	1	00.5)	01.5
Total tested		209	100.0	

After one month of vitamin supplements, there were significant improvements in visual acuity. 61.7% had adequate vision, and only 36.8% had impaired vision (Table 5.2).

Table 4.45: Best corrected visual acuity after 3 months of vitamin supplements

WHO	Best corrected visual acuity	cases	%	
Adequate vision	6/6 or better	72	41.6)	
	can see 6/9	33	19.1)	
	can see 6/12	21	12.1)	
	can see 6/18	21	12.1)	84.9
Visual impairment	Can see 6/24	11	6.4)	
	Can see 6/36	15	8.7)	
	Can see 6/60	0	0)	15.1
Severe visual	Can see 3/60	0	0	
impairment		0	0	
Blind	Cannot see 3/60			
Total tested		173		100.0

Table 4.46: Visual gain of ON cases after 1 month of vitamin supplements

		VA at presentation			
		Adequate vision 6/6 – 6/18	Impaired vision 6/24 - 6/60	S.I/Blind <6/60	Total
VA at 1 month	Adequate vision 6/6 – 6/18	41	86	2	129
	Impaired vision 6/24 6/60		73	4	77
	Severely impaired/Blind < 6/60			3	3
	Total	41	159	9	209

159 (76.01%) cases of those assessed after 1 month of vitamin supplements had recovered part of their visual acuity, while 47 (22.5%) of cases did not continue to lose their visual acuity. Only 3 (1.4%) cases had their visual acuity deteriorated after one month of vitamin supplements.

Table 4.47 Visual gain of ON cases after 3 months of vitamin supplements

		VA at presentation							
		Adequate vision			Impaired vision			S.I/Blind	Total
		6/6 - 6/18			6/24 - 6/60			<6/60	
	Adequate vision 6/6 – 6/18	37			106			4	147
VA at3 months	Impaired vision 6/24 – 6/60				25			1	26
	S.Impaired/Blind < 6/60								
	Total tested	37			131			5	173
	Not tested	2	3	5	9	15	14	4	52
	Total	7	12	28	42	82	45	9	225

158 (91.3%) of cases assessed after 3 months of vitamin supplements had gained in vision, while 15 (8.7%) had maintained their presentation vision. There was no single case that experienced a deterioration of vision, once started on vitamin supplements.

Table 4.48: Colour vision based on 14 Ishihara plates of cases on vitamin supplements

No of plates seen	Presentation		1 month of vitamin supplements		3 months of vitamin supplements	
	No	%	N	%	N	%
14	0		9	4.3	17	9.8
10 – 13	76	33.8	140	67.0	122	70.5
5 – 9	87	38.6	26	12.4	20	11.6
1- 4	60	26.7	33	15.8	14	8.1
Unable to see	2	0.9	1	0.5	0	
Total tested	225	100.0	209	100.0	173	100.0

At presentation only 33.8% of cases were able to see 10-13 Ishihara colour plates. Following vitamin supplements, as the number of cases able to see 10-13 plates increased to 71.3 % after 1 month, and 80.3% after 3 months of follow-up.

Table 4.49: Audiometric Assessment of ON cases on vitamin supplements

Sensori-neural hearing loss		At presentation		1 month F/UP		3 months F/UP	
		N	%	N	%	N	%
No loss		64	34.0	135	81.8	137	90.1
Mild loss	20 – 30 dB loss	83	44.2	7	4.2	7	4.6
Moderate loss	40 – 59 db loss	40	21.3	11	6.7	8	5.3
Severe loss	> 60 dB loss	1	0.5	12	7.3		
Total tested		188	100.0	165	100.0	152	100.0

At presentation, only 34% of cases had normal hearing tests on audiometry. 81.8% cases had normal tests after 1 month , and 90.1 were tested normal after 3 months of vitamin supplements.

Table 4.50: Neurological assessment of ON cases on vitamin supplements

Signs of peripheral neuropathy	At presentation		1 month F/UP		3 months F/UP	
	N	%	N	%	N	%
No signs	145	64.7	128	62.1	124	73.0
Signs of p. neuropathy	79	35.3	78	37.9	46	27.0
Total tested	224	100.0	206	100.0	170	100.0

Based on assessment of tandem walking, heel-shin ataxia, vibration sensation at ankles, cotton wool sensation in lower limbs, knee and ankle jerk and joint position awareness.

Chapter 5

Discussion, Conclusions & Recommendations

5A: Prevalence studies:

5A1: Tanzanian prevalence study

On the basis of a prevalence of disease of 2.4 percent (95% confidence interval of 1.7 to 3.0 per cent) and the patterns of presentation at health clinics, we estimate that there were 40 000 persons were affected with optic neuropathy in Dar es Salaam at the time of this study. Not included in this study are people with isolated peripheral neuropathy, who seek care at primary health clinics with the same frequency as those with optic neuropathy.

Our estimate is that 80 000 persons in Dar es Salaam have the disease, of whom:

- 25 000 persons have pure optic neuropathy
- 40 000 persons have pure peripheral neuropathy
- 15 000 persons have both optic and peripheral neuropathy

This disease mainly affects persons aged 10 – 40 years. Groups at particular risk of disease are: adolescent youth, pregnant women, lactating women and the very poor. The strongest known risk factor is breast-feeding (relative risk: 12.5).

The cause of the epidemic is unknown. Cyanide intoxication from improperly processed cassava and tobacco smoking have been ruled out (Plant et al., 1997).Mitochondrial DNA analysis have not shown any common mutations associated with Leber's hereditary optic neuropathy (Plant et al., 1997).

The disease is clinically identical to the recent epidemic of bilateral optic neuropathy seen in Cuba (the Cuba Neuropathy field Investigation team 1995). That epidemic resulted in more than 50 000 patients (Mission to Cuba PAHO/WHO 1993).

Our survey data suggests that the Tanzanian epidemic neuropathy is the largest outbreak of epidemic neuropathy ever reported. The social and economic consequences of the disease are enormous. Persons with the disease, if left untreated, are unlikely to be able to undertake any form of employment or education for the remainder of their lives. With an estimated 80 000 affected persons, the epidemic must be having a devastating impact on the meagre community health services. This is likely to lead to overloading of the health services, and a drain on their already limited resources.

5A2: Gambian Prevalence study

Clinical review of bilateral optic neuropathy cases identified in the 1996 national survey of blindness in the Gambia identified 4 epidemic optic neuropathy cases. These are non-epidemic cases and indicate background rates in a non-epidemic situation.

The prevalence of epidemic optic neuropathy in the Gambia was 0.05 %(95% confidence interval, 0.01 to 0.05%), compared to 2.4% in Dar es Salaam. This means that the prevalence in Dar es Salaam is 48 times higher than in the Gambia.

The clinical characteristics of the Gambian epidemic optic neuropathy cases are very similar to the Tanzanian cases, except that the Gambian cases seem to be older than the Tanzanian ones.

This study provides, for the first time, a detailed description of cases of optic atrophy and neuropathy as they occur in an unbiased random sample of a population. Undoubtedly, it enlarges the clinical understanding of optic neuropathies in general, plus provides a unique data on their prevalence in a coastal West African country

5B- Case series studies:

These studies provide a clinical description of the acute stage of the epidemic neuropathy in Tanzania and Somalia. Our design, using fundoscopic abnormalities, plus field defects to define case-patients status and excluding any other patient, gave a rigorous assignment of case patient.

The rapid referral system was successful in identifying acute cases with recent onset of visual loss in Tanzania. Several cases presented to the clinic within 2 days of disease onset.

Ophthalmic findings

The prominent clinical features were bilateral loss of visual acuity with central or centro-caecal scotomas and diminished colour vision. The optic discs were either normal, or with dilated capillaries and blurred margins in the early acute cases. These are classic signs of acute inflammation of the optic nerve. After four weeks virtually all cases (99%) had progressed to temporal pallor of the disc, and loss of papillo-macular bundle. This is consistent with previous reports on chronic cases (Plant et al., 1997).

Neurological disorder

We found a higher prevalence of signs of peripheral neuropathy among the studied cases than in earlier studies (Plant et al., 1997, Johnson e al., 1991).

52% of Tanzanian cases and 59% of the Somalian cases reported having burning sensations or numbness at the lower limbs. Neurological assessment indicated that 35% had signs of peripheral neuropathy at presentations in Tanzania.

These were mainly increased vibratory and thermal thresholds in the legs, suggesting the involvement of small nerve fibres in addition to the large myelinated fibres most often involved in toxic or metabolic neuropathies. (Plant et al., 1997).

Auditory symptoms and signs

Symptomatic hearing loss had occurred in 31.5% of Tanzanian cases and 28% of Somalian cases . Audiometric assessment in Tanzania revealed that 66% of patients had sensorineural hearing loss. This indicates that over 34% of patients had asymptomatic hearing loss associated with the optic neuropathy.

Other features

Virtually all cases were from low income housing areas. All cases did not have treatment prior to the study. Because of this, together with the range of intervals between onset of disease and presentation, the baseline presentation data provides a cross-sectional view of the natural history for the first 12 weeks of the disease, in the absence of any intervention. Greatest loss of vision occurs during the first 2-4 weeks, without treatment, visual acuity continued to decline over the first 8 weeks of the disease process. Multivariate analysis showed that there were no predictors of disease severity at presentation. This disease is clinically identical to the Cuban optic neuropathy (Sadun et al., 1994; Thomas et al., 1995). The age distribution in the Tanzanian epidemic and in Somalia , however, is distinct as a younger age group has been affected than in the Cuban epidemic (Mean age was: Tanzanian cases : 25.5 years, Somalian cases: 24 years, while for the Cuban cases, it was 45 years) (The Cuba neuropathy field investigation team 1995). Other major differences between the Tanzanian and Somalian cases versus the Cuban cases were, the fact that smoking was widespread among the Cuban patients (83% of them smoke cigarettes), compared to 13.3% of smokers among the Tanzanian cases and 9.5% among the Somalian cases. A very common feature in the Cuban epidemic has been weight loss (10 – 20 Kg) coincident with the onset of symptoms (Thomas et al., 1995), 44.7% of Somalian cases and only 21.3% of the Tanzanian patients had reported weight loss.

The peripheral neuropathy appears to develop either slowly or at a later stage to the optic neuropathy. This suggests the peripheral neuropathy may have a slightly different, but probably overlapping aetiology.

The present epidemic has many features which overlap with previous reports of optic neuropathy, peripheral neuropathy and deafness occurring in the context of poor nutrition usually in the tropics (Strachan's syndrome). Apart from the association with poor nutrition the aetiology of the disorder is unknown.

Epidemics of neuropathy have been reported primarily during wartime, especially among prisoners of war in Asia during World War II (Spillane JD 1947). An epidemic in which clinical abnormalities were identical to those in the 1991-1993 Cuban epidemic was reported in Havana during the Spanish-American war (Madan D 1898). Although the cause of these earlier epidemics was never established, contemporary neurologists linked them to dietary deprivation and lack of B-complex vitamins (Spillane JD 1947), and Tobacco use was implicated in optic neuropathy among prisoners of war (Knox et al., 1982).

In Africa, exposure to cyanide from cassava consumption has been implicated in epidemics of neurological disease. However, unlike those in this Tanzanian epidemic, affected subjects have usually had spastic paraparesis (Mozambique, Ministry of Health 1984). There is greater overlap with Tropical Ataxic neuropathy (TAN) which has been attributed to chronic cassava consumption (Osuntokun, 1981), but the present study shows low levels of cassava consumption in the cases, as only 20% of cases consume it more than three times a week.

Although we cannot assign a definitive cause to this epidemic, our findings suggest that its occurrence was linked to deterioration in diet affecting vitamin B nutrients.

5C- Etiological Studies:

5C1: Cyanide intoxication:

These results are within the range seen in healthy individuals; they are similar to the results obtained in chronic cases and controls previously (Plant et al., 1997).

These results should be compared with published mean serum and urine thiocyanate levels of 307 and 904 μmol/L respectively in a Zairian population which is consuming a high dietary proportion of bitter cassava. This population was affected by the spastic paralysis attributed to cyanide intoxication known as konzo. (Tylesskär et al., 1992).

The suggestion that cyanide intoxication may be implicated in the pathogenesis of optic neuropathy in some cases had been repeated in literature with reference to both smoking and cassava consumption. Indeed both cassava consumption and smoking were found to be risk factors in the Cuban epidemic (The Cuban Optic Neuropathy Field Investigation Team, 1995). Very few young Tanzanians smoke tobacco but the consumption of cassava in the city has increased in recent years.

The strength of this study is that we were working on acute cases with recent onset of disease. We now confirm beyond any doubt that the population at risk of developing epidemic optic neuropathy in Tanzania is subjected to very low levels of cyanide exposure from all sources and that there is no evidence of acute exposure associated with disease onset.

5C2: Leber's hereditary optic neuropathy:

We know of no information in the literature suggesting that black African populations may harbor different mutations from those seen in Caucasians, the evidence from Cuban black and Caucasian populations is to the contrary (Torroni et al., 1995).

Studies in Cuban optic neuropathy cases found that many patients with LHON, which started at the time of the epidemic , were incorrectly classified as suffering from this, and perhaps their condition worsened due to the toxic nutritional features common to both conditions (Santiesteban et al., 1995).

Mitochondria DNA can be impaired either genetically (as in Leber's) or through acquired insults (such as nutritional or toxic factors). Either may challenge energy production in all cells of the body (Sadun A 1995).

Our results show with great rigor that common mitochondrial DNA mutations seen in LHON are not risk factors for the development of Tanzanian Epidemic Optic Neuropathy. The most common mitochondrial DNA mutations associated with LHON did not appear to be contributing factors in the epidemic neuropathy in Cuba (Newman et al., 1994; Torroni et al., 1995).

5C3: Vitamin status of Tanzanian ON cases and controls

Nutritional deficiency of vitamins results in inhibition of key metabolic pathways. Deficiencies in the production of important enzymes, coenzymes (such as FAD, FMN and ATP) or compound carriers play a role in the starvation of metabolic fuel from the nerve cells, resulting in the so called "neural metabolic lesion". There exists in neurons a threshold which, once passed, leads to catastrophic changes. This threshold may be that point at which mitochondrial derangement leads to such ATP depletion that axonal transport is compromised. Neurons are singularly dependent on the axonal transport of mitochondria (Sadun A 1995).

The presence of riboflavin deficiency among both cases and controls suggests that riboflavin deficiency on its own is not sufficient to produce the optic neuropathy. Riboflavin deficiency may be part of a multi-factorial aetiology.

The clear deficiency in retinol and tocopherol levels of the Tanzanian samples compared to the British samples in the NDNS study are likely due to either: differences in total plasma lipids,, especially for the tocopherols; age differences, especially for retinol; possibly differences in acute phase status, since the acute phase reaction lowers plasma retinol levels; Possibly differences in dietary intakes of these nutrients.

The higher lutein and lycopene levels in the Tanzanian subjects are, however, quite likely to be diet-related, because these carotenoids are fairly good markers of carotenoid, and hence vegetable intakes and they are not converted to vitamin A in the body.

This study is the first time that acute cases have been tested for micronutrient deficiency, and is the first clear demonstration of an identifiable abnormality which may be of etiological significance.

Identical Thiamine (B_1) and Riboflavin (B_2) deficiencies among cases and controls, suggests that a B-complex deficiency on its own is not sufficient to produce the neuropathy. Measurements of urinary thiamine and blood transketolase and its activation with thiamine pyrophosphate were made in Cuban ON cases and controls (Macias-Motos et al., 1995). The Cuban samples showed a high prevalence (30-70%) of thiamine depletion. Severity of biochemical depletion was, however, no greater in the neuropathy subjects than in the control subjects ($P > 0.05$). Studies in Cuba confirmed the high prevalence of poor B-vitamin status and have produced new evidence about the complex seasonal cycles of micronutrient availability and biochemical status in men living in Havana (Arnaud et al. 2001 a,b).

They have also demonstrated a dramatic deleterious effect of smoking on riboflavin status and on some (but not all) classes of serum carotenoids, and a smaller but significant effect on total serum protein (but not serum albumin) (Barnouin et al., 2000).B-complex deficiency is part of a multi-factorial aetiology.

Nutritional shortages that lead to a reduction of folates, and the intake of small amounts of methanol in alcoholic drinks could lead to a lacking energetic states which would facilitate that the optical nerve be affected and the epidemic optical neuropathy appear in Cuba (Elles et al., 2000). In contrast the Tanzanian samples had normal folate concentration, and were not consumers of alcohol drinks.

Vitamin B_{12} serum levels did not differ significantly between cases and controls, and they had normal serum concentration. In Cuba it was hypothesized that vitamin B_{12} participated to the etiological pathway through its cyanide detoxification capacity, but the vitamin was not a precipitating factor (Barnouin et al., 2000).However, if an insult such as exposure to cigarette smoke were to alter the tissue distribution or balance of coenzyme and/or non coenzyme forms of the vitamin, then a functional deficiency at a critical site might arise even in the presence of an apparently normal serum concentration (Chisholm et al., 1967). The implication is that we may need to develop more discriminatory biochemical indices in order to resolve such uncertainties.

There was no difference of fat soluble vitamins levels among the Tanzanian cases and controls. The Tanzanian samples were deficient in retinol and tocopherol levels compared to British samples. It is well known that carotenoids have antioxidant roles to defend neural cells against toxic agents. Although levels of toxic metabolite of cassava were not raised in the studied cases, it may be that a similar mechanism is operating here, i.e. A deficiency of one dietary component leading to defective handling of another.

Nearly all circumstantial clues seem to point in the direction of malnutrition as being an important component of the risk complex, with a number of micronutrient deficiencies being on the list of suspects. In the Cuban epidemic widespread distribution of vitamin supplements led to a virtual cessation of new cases .

Whether the B-vitamins with their coenzyme roles, or nutrients such as specific carotenoids and amino acids with their antioxidant roles, are the prime suspects, is still a matter for speculation.

5D: Treatment study:

Prior to this study, our understanding of the Tanzanian epidemic optic neuropathy was largely based on chronic cases examined many months after disease onset. Nothing was known about the benefits of B-complex supplement on disease prognosis.

There were significant improvements in visual acuity. 76% and 91% of cases had gained vision at 1 month and 3 months follow-up respectively. At 3 month follow-up, only 9% of cases had the same visual acuity they had at presentation, and there was no single case with vision deterioration at this stage.

There were similar improvements in colour vision, as the proportion of cases able to read 10 or more Ishihara plates were 34%, 67% and 70% at presentation, 1 month and 3 months after vitamin supplements.

Earlier studies from Tanzania have documented that without intervention, only 10 – 20 of cases have spontaneous recovery (Johnson et al 1993).

66% of cases presented with at least 20 dB broad spectrum loss of sensorineural hearing loss. After 1 month of vitamin supplements, only 18.2% of cases had sensori-neural hearing loss, and at three months follow-up 9.9% of cases had sensor-neural hearing loss on audiometry. This is indeed a significant improvement of the hearing sense. Neurological assessment indicated that 35.5% of cases had signs of peripheral neuropathy at presentation. At the one month follow-up clinic, the proportion of cases with signs of peripheral neuropathy had increased to 37.9%. This increase was due to 27 cases developing neurological signs after presentation to the clinic. At the 3 month follow-up, the proportion with signs of peripheral neuropathy had decreased to 27%. The peripheral neuropathy appears to develop either slower or at a later stage to the optic neuropathy.

All cases received no treatments or medicines between disease onset and presentation to the clinic. Vitamin supplements have resulted in substantial visual recovery for over 90% of the Tanzanian epidemic optic neuropathy.

In Cuba a temporal association was noted between the decline in cases and the distribution to the entire population of Cuba of a vitamin supplement containing B-group vitamins, folate, and vitamin A. Distribution began in the first week of May, 1993. During the last semester of 1993 incident cases gradually abated and by the years end the epidemic vanished.(Roman et al., 1994).

5 E: Conclusions and Recommendation for future studies:

The epidemic of optic neuropathy is still ongoing in Dar es Salaam, Tanzania (personal communication), and there are signs of the disease in Mogadishu, Somalia.

This epidemic has very strong similarity to the Cuban epidemic of optic neuropathy that occurred in early 90s, and our conclusion is that they are the same entity.

This study has demonstrated that the epidemic is treatable with vitamin B-supplements.

The fact that cases did not differ from controls with respect to many of the nutritional factors explored, can be explained by "Rose hypothesis" outlined in his paper "sick individuals and sick populations" as large sectors of the population may be deficient (explaining the high incidence of disease) (Rose 2001).

Aetiology confronts two distinct issues: the determinants of individual cases, and the determinants of incidence rate. If exposure to a necessary agent is homogeneous within a population, then case/control and cohort methods will fail to detect it.

The community's immediate needs to cope with this huge outbreak are:

- training of community eye care workers on how to diagnose the disease,
- establish a surveillance system in community health clinics to provide data on patterns of disease occurrence and to monitor changes in the epidemic pattern,
- develop guidelines for the health services for treating existing cases and preventing new cases from occurring,
- Initiate community intervention strategies, with a mass distribution of vitamin B-complex supplements to halt the epidemic.

Cystine is an amino acid that exists in both oxidized (cystine) and reduced forms. Plasma contains mainly the oxidized form, while the major cellular form of the compound is the reduced form cysteine. This compound is utilized intracellulary for a variety of metabolic roles, most specifically for the biosynthesis of gluatathione. Glutathione is a major cellular antioxidant and exists in neural tissues at considerable concentrations. There is compelling evidence that plasma-borne cystine is the primary source of intracellular cysteine. Thus a deficiency of plasma cystine would be expected to result in a deficiency not only of intracellular cysteine itself, but chronically to result in a deficiency of cysteine-derived metabolic products. It is likely that the most important of these products would be glutathione.

Preliminary data from analysis of the free amino acids of blood plasma (cases= 14,controls=14), revealed that samples from the diseased subjects were seriously deficient in the cystine and that this effect was specific to this amino acid alone. The content of methionine in all the plasma samples tested was lower than would be expected from subjects consuming western diets.

Low plasma methionine concentration suggests that all subjects whose plasma was analyzed in this study had previously consumed diets that were deficient in the sulphur-containing amino acids (methionine and cysteine).

The deficiency in cystine in the plasma samples from those affected subjects is enormous, even greater than plasma samples in experiments in which diets deficient in sulphur amino acids were consumed by human volunteers. A number of mechanisms might bring about this situation:

a- Much methionine (perhaps as much as 90%) is converted to cystine + cysteine *in vivo*, and this material will contribute to the plasma concentration of cystine. Hence a dietary compound that inhibited this conversion could lead to a diminution in the concentration of plasma cystine.

b- Food-derived compounds could react with plasma cystine directly and cause the observed removal of cystine from plasma. Such substances include cyanide and sulphite.

It has previously been demonstrated that deficiency of amino acids in the blood can lead to defective detoxification after cassava consumption (Tor-Agbidye et al., 1998).The association between high cyanide and low sulphur intake in cassava-induced spastic paraparesis has been demonstrated (Cliff et al., 1985).

As the amino acid content of plasma is directly related to the food intake, it would be appropriate to analyse amino acid plasma levels immediately after a meal.

The study in Mogadishu did not have the aetiological component, as political situation there did not permit for the transportation of urine and blood samples to London. But there is a great hope that the political situation in Mogadishu, improves in the near future.

Further studies are needed to ascertain amino acid levels of cases and control, either from Tanzania or Somalia. Clinical trials involving two alternative treatments will be needed to further analyse the impact of vitamin supplements to this disease.

References

Alfaro, I. And Gonzalez-Quevedo, a. (1994) Immunoglobulins in epidemic neuropathy in Cuba. J. Neurol. Sc., in press

Arias L, Echemendia E (1993): Neuropatia epidemica en Cuba: alteracions auditivas. Informe preliminar Hospital "Dr. Luis Diaz Soto", Guidad de la Habana, pp 1-6.

Arnaud J, Fleites-Mestre P, Chassagne M, Verdura T, Garcia Garcia I, Hernandez-Fernandez T, Gautier H, Favier H, favier A, Perez-Cristia R, Baronouin J (2001): Vitamin B intake and status in healthy havanen men, 2 years after the Cuban neuropathy epidemic. Br j Nutr. 85(6): 741-8

Barnouin J, Perez Cristia R, Chassagne M, barrios TV, Arnaud J, Fleites Mestre P, favier A (2000): vitamin and nutritional status in Cuban smokers in the context of an emerging epidemic neuropathy. Int J Vitam Nutr Res. 70 (3) 126-38

Berger JR, Sheremata WA, Resnick L, Atherton S, Fletcher MA,Norenberg M. (1989): Multiple sclerosis-like illness occurring with human immunodeficiency virus infection. Neurology 39:324-9

Borrajero, I., Perez, J.L., Dominguez, C., Chong, A., Coro, R.M., Rodriguez, H., Gomez, N., Roman, G.C. and Navarro-Roman, L., (1994): Epidemic neuropathy in Cuba: morphological characterization of peripheral nerve lesions in sural nerve biopsies. *J. Neurol. Sci.* 127, pp. 68–76

Burton BJL, leff AP, Plant AT. (1998):Steroid-Responsive HIV Optic Neuropathy. Journal of Neuro-Ophthalmology 18(1): 25-29

Carelli V, Ghelli A, Ratta M, *et al.* (1997):Leber's hereditary optic neuropathy: biochemical effect of 11778/ND4 and 3460/ND1 mutations and correlation with the mitochondrial genotype. *Neurology* 48:1623–32.

Carelli V, Ghelli A, Bucchi L, *et al.* (1999): Biochemical features of mtDNA 14484 (ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. *Ann Neurol* 45:320–8.

Carroll F.D., (1971): Jamaican optic neuropathy in immigrants to the United States. *Am. J. Ophthalmol.* 71, pp. 261–265 -

Casadei E., Cliff, J. and Neves, J (1990): Surveillance of urinary thiocyanate concentration after epidemic spastic paraparesis in Mozambique. *J. Trop. Med. Hyg.* 93, pp. 257–261

Cabrera J, Collazzo h, Garcia F et al (1993): Caracteristicas clinicas de la neuropatia epidemica en la forme periferica: reporte initial. Hospital provincial clinico quirurgico Dr. Adereguia y Hospital Pediatrico Paquito Gonzalez, Crenfuegos, Cuba, pp 1-23.

Carton, H., Kayembe, K., Odio, Kabeya, Billian, A. and Maertens, K (1986): Epidemic spastic paraparesis in Bandundu (Zaire). *J. Neurol. Neurosurg. Psychiatry* 49, pp. 620–627

Chalmers RM, Schapira AHV. Clinical biochemical and molecular genetic features of Leber's hereditary optic neuropathy. *Biochin Biophys Acta* 1999;1410:147–58

Chisholm I, Bronte-Stewart J and Foulds W (1967): Hydroxycobalmin versus cyanocobalmin in the treatment of Tobacco Amblyopia. Lancet; 2: 450-51

Cliff J (1985): Association of high cyanide, and low sulphur intake in cassava induced spastic paraparesis. Lancet,ii:1211-1213.

Compston A, Ebers G, Lassmann H, McDonald J, Matthews PM, Wekerle H. . (1998): McAlpine's multiple sclerosis. 3rd edLondon: Churchill Livingstone.

Cortelli P, Montagna P, Avoni P, Sangiorgi S, Bresolin M, Moggio M, Zaniol P, Mantovani V, Barboni P, Barbiroli B, Ingaresi E (1991): Leber's hereditary optic neuropathy: Genetic, biochemical, and phosphorus magnetic resonance spectroscopy study in an Italian family. Neurology 41:1211-1215.

Crews S.J (1963): Bilateral amblyopia in West Indians. *Trans. Ophthalmol. Soc. U.K.* 83, pp. 653–667

Cruickshank EK (1946): painful feet in prisoners of war in the Far East: review of 500 cases. Lancet 2: 369-72

Cruickshank EK (1976): Effects of malnutrition on the central nervous system. Handbook clin. Neurology. 28: 1-41

Degazon, D.W (1956): Tropical amblyopia in Jamaica. *W.I. Med. J.* 5, pp. 223–230

Egger J, Wilson J (1983): Mitochondrial inheritance in a mitochondrial mediated disease. N Engl J Med 309:142-146.

Elles JT, Gonzalez-Quevedo A, Santiesteban R, McMartin KE, Sadun AA (2000): Folic acid deficiency and increased concentrations of formate in serum and cerebrospinal fluid of patients with epidemic optical neuropathy. Rev Cubana Med trop. 52(1): 21-3

Erickson RP (1972): Leber's optic atrophy: A possible example of maternal inheritance. Am J Hum Genet 24:348-349.

Espinosa A, Apoliinaire J, Alvarez O (1993): Alcona considerations sobre un brote de neuropatia epidemica en la recluourio de Arizo, Provincia de Creinfuegosa. Rev. F. Creinfuegosa, pp 2-3.

Estrada R, Nunez H, Centron I (1993): Informe sobre la visita realizada al hospital "Saturnino Lora" de Santiago de Cuba para evaluacion de syndrome neurological de reciente presentacion. Instituto de Neurologia y Neuroqirurgica,, Ministerio de Salud Publica, Republica de Cuba, pp. 1-3.

Faal H, Minassian D, Dolin P, Mohamed A et al (2000): Evaluation of a national eye care programme: re-survey after 10 years Br J Ophthalmol; 84: 948-951

Fasler, J.J. and Rose, F.C (1980): West Indian amblyopia. *Postgr. Med. J.* 56, pp. 494–500

Finch S, Doyle W, Lowe C, *et al.* (1998): National diet and nutrition survey: people aged 65 years and over. Volume1: Report of the diet and nutrition survey. London: Stationery Office,

Fisher, C.M (1955): Residual neuropathological changes in Canadians held prisoners of war by the Japanese. *Can. Serv. Med. J.* 11, pp. 157–164

Funakawa I, Kato H, Terao A, Ichihashi K, Kawashima S, Hayashi T, Mitaffi K, Miyazaki S (1995): Cerebellar ataxia in patients with Leber's hereditary optic neuropathy. J Neurol 242:75-77

Gill GV, Bell DR (1982): Persisting nutritional neuropathy amongst former war prisoners. J Neurol Neurosurg Psychiatry 45: 861-5

Haddock, D.R.W., G.J. Ebrahim and B.B. Kapur (1962) Ataxic neurological syndrome found in Tanganyika. Br. Med. J., ii: 1442–1443.

Harding AE, Sweeney MC, Govan GG, Riordan-Eva P (1995): Pedigree analysis in Leber hereditary optic neuropathy families with a pathologic mtDNA mutation. Am J Hum Genet 57:77-86.

Hirano M, Cleary JM, Stewart AM, Lincoff NS, Odel JG, Santiesteban R, Santiago Luis R (1994): Mitochondrial DNA mutations in an outbreak of optic neuropathy in Cuba. Neurology 44:843--845.

Howell N. Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. *Vision Res* (1997):37:3495–507.

Howlett, W.P., Brubaker, G.R., Mlingi, N. and Rosling, H. (1990): Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain* 113, pp. 223–235

Huoponen K (2001): leber hereditary optic neuropathy: clinical and molecular genetic findings. Neurogenetics 3:119-25

Johns, D.R., Neufeld, M.J. and Hedges, T.R., 1994. , Mitochondrial DNA mutations in Cuban optic and peripheral neuropathy. *J. Neuro-Ophthalmol.* 14, pp. 135–140

Johnson, G.J., Mtanda, A.T. and Negrel, A.-D., (1991): Macular degeneration of unknown origin in Tanzania. *Lancet* 338, pp. 827–828

Johnson, G.J., Mtanda, A.T., Kinabo, N.N., Sangawe, J.L.F., Masesa, D.E. and Negrel, A.-D., (1994): Optic nerve and macular atrophy of unknown origin in Tanzania. *Arch. Publ. Hlth.* 51, pp. 561–571

Knox D, Chen M, Guilaite T et al (1982): Nutritional Amblyopia: Folic Acid, Vitamin B12 and other Vitamins, Reta 2: 288-292.

Larsson NG, Anderson 0, Holme E, Oldfors A, Wahlstrom J (1991): Leber's hereditary optic neuropathy and complex I deficiency in muscle. Ann Neurol 30:701-708.

Latham, M.C., (1964): Nutritional aetiology of a neuropathy found in Tanganyika. *Br. J. Nutr.* 18, pp. 129–134

Lincoff N, Odel J, Hirano M (1993): Letter from Havana: "Outbreak" of optic and peripheral neuropathy in Cuba? JAMA; 270: 511-518.

Ludolph A, Hagon J et al (1987): Studies on the aetiology and pathogenesis of motor neurosis 1. Lathyrism: Clinical findings in established cases. Brain; 110 (pt 1) 149-65.

Lundquist, P., Mårtensson, J., Sörbo, B. and Öhman, S., (1979): Method for determining thiocyanate in serum and urine. *Clin. Chem.* 25, pp. 678–681

Macias-Motos C, Rodriguez-Ojee A et al (1996): Biochemical evidence of thiamine depletion during the Cuban neuropathy epidemic, 1992-1993. Am J Clin Nutr; 64 (3): 347-53

Mackey DA (1994): Three subgroups of patients from the United Kingdom with Leber hereditary optic neuropathy. Eye 8:431-436.

Madan, D., (1889): Notas sobre una forma sensitiva de neuritis periferica ambliopia por neuritis optica retrobulbar. *Crónica Médico-Quirurgica de la Habana* 24, pp. 81–86

Majander A, Huoponen K, Savontaus ML, Nikoskelainen EK, Wikstrom M (1991): Electron transfer properties of NADH: ubiquinone reductase in the ND1/3460 and the ND4/11778 mutations of Leber hereditary optic neuroretinopathy (LHON). FEBS Lett 292:289-292.

Malessa R, Agelink MW, Diener H. (1995): Dysfunction of visual pathways in HIV-1 infection. J Neurol Sci: 130:82-7

Mas Bermejo P, Rodriguez A et al (1993): Neuropatia epidemica en Cuba: Un analisis epidemiologico, Ministerio de la Salud Publica, la Habana, pp. 1-14.

Mlingi, N., Kimatta, S. and Rosling, H., (1991): Konzo, a paralytic disease observed in southern Tanzania. *Trop. Doct.* 21, pp. 24–25

Ministry of Health, Mozambique (1984): Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bull WHO; 62:477-484

Mission to Cuba PAHO/WHO (1993): Epidemic Optic Neuropathy in Cuba. PAHO Epidemiol. Bull.; 14: 1-4.

Mojon, D.S., Kaufmann, P., Baker, H., Lincoff, N.S., Odel, J.G. and Hirano, M., (1996): Vitamin status and mitochondrial DNA mutations in patients with incomplete recovery from an epidemic optic neuropathy in Cuba. *Neuro-ophthalmology* 16, pp. 355–360 -

Monekosso G. (1964) Clinical, epidemiologic observations on an ataxic syndrome in western Nigeria. *Trop Geogr Med*; 4:316-323

Money G. (1958) Endemic neuropathies in the Epe district of southern Nigeria. *West Afr Med J*; 7:58-62

Money, G.L., (1959): Clinical Aspects of tropical ataxic neuropathies related to malnutrition. *West Afr. Med. J.* 8, pp. 3–17

Moore D. (1930) Partial loss of central acuity of vision for reading and distance in school children and its possible association with food deficiency. *West Afr Med J*; 3:46-51

Moore D. (1937) Nutritional retrobulbar neuritis followed by partial optic atrophy. *Lancet*: 1225-1227.

Moore D. (1939) Retrobulbar neuritis with pellagra in Nigeria. *J Trop Med Hyg*; 42:109-114.

Moore D. (1940) Pellagra or pellagra-like conditions in association with deficiency of vitamin A. *J Trop Med Hyg*; 42:190-194

Mtanda, A.T., Plant, G., Arden, G.B. and Johnson, G.J., (1994): The Tanzanian epidemic of subacute retrobulbar neuropathy .*Ophthalm. Epidemiol.* 1, pp. 53–54

Mwanza JC, Nyambo LK, Tyleskär T, Plant GT (2003): Visual evoked potentials on Konzo, a spastic paraparesis of acute onset in Africa. Ophthalmologica, 217;381-6

Mwanza JC, Tshala-Katumbay D, Kayembe DL, Eeg-Olofsson KE, Tylleskar T. (2003): Neuro-ophthalmological findings in konzo, an upper motor neuron disorder in Africa. Eur J Ophthalmol May; 13 (4): 383-9

Mwanza JC, Nyambo LK, Tyleskär T, Plant GT: Neuro-ophthalmologic disorders in HIV-infected subjects with neurologic manifestations (PhD thesis) 2004

Newman NJ, Lott MT, Wallace DC (1991): The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. Am J Ophthalmol 111:750-762

Newman NJ, Lessell S. (1992): Bilateral optic neuropathies with remission in two HIV-positive men. J Clin neuro Ophthalmol 12:1-5

Newman NJ, Torroni A, Brown MD, Lott MT, Femandez MM, Wallace DC (1994): Epidemic neuropathy in Cuban no associated with mitochondrial DNA mutations found in Leber's hereditary optic neuropathy patients. Am J Ophthalmol 118:158-168

Nikoskelainen E, Hoyt WF, Nummelin K (1982a): Ophthalmoscopic findings in Leber's hereditary optic neuropathy. I. Fundus findings in asymptomatic family members. Arch Ophthalmol 100: 1597-1602.

Nikoskelainen E, Hoyt WF, Nummelin K (1982b): Ophthalmoscopic findings in Leber's hereditary optic neuropathy. II. Fundus findings in affected family members. Arch Ophthalmol 101:1059-1068.

Nikoskelainen E, Hassinen IE, Paljarvi L, Lang H, Kalimo H (1984a): Leber's hereditary optic neuroretinopathy, a mitochondrial disease? Lancet 2:1474.

Nikoskelainen E, Hoyt WF, Nummelin K, Schatz H (1984b): Fundus findings in hereditary optic neuroretinopathy. III. Fluorescein angiographic studies. Arch Ophthalmol 102:981-989.

Nikoskelainen E, Savontaus ML, Wanne OP, Katila Mj, Nummelin KU (1987): Leber's hereditary optic neuroretinopathy, a maternally inherited disease. A genealogic study in four pedigrees. Arch Ophthahnol 105:665-671.

Nikoskelainen EK, Marttila RJ, Huoponen K, Juvonen V, Lamniinen T, Sonninen P, Savontaus ML (1995): Leber's "plus": Neurologic abnormalities in patients with Leber's hereditary optic neuropathy. J Neurol Neurosurg Psychiatry 59:160-164.

Nikoskelanien EK, Huoponen K, Juvonen V, Lamminen T, Nummelin K, Savontaus ML (1996): Ophthalmic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations. Ophthalmology 103:504-514.

Oluwole OSA, Onabolu AO, Hans Link, Hans Rosling (2000): Persistence of Tropical ataxic neuropathy in a Nigerian community. J Neurol, Neurosurgery, Psychiatry ; 69: 96-101

Onabolu A, Bokanga M, Rosling H. (1999) Cassava processing in a Nigerian community affected by a neuropathy attributed to dietary cyanide exposure. *Tropical Science*; 39:129-135.

Oostra RJ, Bolhuis PA, Wijburg FA, Zom- Ende G, Bleeker-Wagemakers EM (1994): Leber's hereditary optic neuropathy: Correlations between mitochondrial genotype and visual outcome. J Med Genet 31:280-286.

Osuntokun BO. (1968) An ataxic neuropathy in Nigeria. A clinical, biochemical and electrophysiological study. *Brain*; 91:215-248

Osuntokun, B.O., Monekosso, G.L. and Wilson, J., (1969): Relationship of a degenerative tropical neuropathy to diet. Report of a field survey. *Br. Med. J.* 1, pp. 547–550

Osuntokun BO, Langman MJ, Wilson J, *et al.* (1970) Controlled trial of hydroxocobalamin and riboflavine in Nigerian ataxic neuropathy. *J Neurol Neurosurg Psychiatry*; 33:663-666

Osuntokun, B.O. and Osuntokun, O., (1971): Tropical amblyopia in Nigerians. *Am. J. Ophthalmol.* 72, pp. 708–716

Osuntokun B, Langman M, Wilson J, *et al.* (1974) Controlled trial of combinations of hydroxocobalamin-cysteine and riboflavine-cysteine, in Nigerian ataxic neuropathy. *J Neurol Neurosurg Psychiatry*; 37:102-10

Osuntokun, B.O., (1981): Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World Rev. Nutr. Diet.* 36, pp. 141–173

Osuntokun B. (1994) Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. *Acta Horticulturae*; 375:271-283

Owen, R.A., (1966): Defective vision in West Indian immigrants. *Br. J. Ophthalmol.* 68, pp. 561–569

Plant, G.T., (1995): , Recent advances in the electrophysiology of visual disorders. *Curr. Opinion Ophthalmol.* 6, pp. 54–59

Plant, G.T. and Perry, V.H., (1990): The anatomical basis of the caeco-central scotoma: new observations and a review. *Brain* 113, pp. 1441–1458

Plant, G.T., Mtanda, A.T., Arden, G.B. and Johnson, G.J., (1997): An epidemic of optic neuropathy in Tanzania: Characterization of the visual disorder and associated peripheral neuropathy. *J. Neurol. Sci.* 145, pp. 127–140

Plant GT, Dolin P, Mohamed AA, Mlingi N. (1997): Confirmation that neither cyanide intoxication nor mutations commonly associated with Leber's Hereditary Optic Neuropathy are implicated in Tanzanian Epidemic Optic Neuropathy. J Neurol Sci. 152, pp 107-8

Peraita, M., (1938): Deficiency neuropathies in Madrid during the civil war. *Br. J. Ophthalmol.* 23, p. 784

Perez J, Nasiff a, et al (1993): Neuropatia epidemica en Cuba: Caracterizacion clinica en 62 casos. Hospital clinico quirurgico "Hermanos Amerijeiras",la Habana, pp 1-24

Ramirez A Rodriguez C, et al (1993): Neuropatia epidemica; breve resena epidemiologica. IPK Boletin epidemiologico, Numero especial, 1-5

Riordan-Eva P, Sanders MD, Govan GG, Sweeney MC, Da Costa J, Harding AE (1995): The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation. Brain 118:319-337

Rodgers-Johnson, P.E., Garruto, R.M. and Gajdusek, D.C., (1988): Tropical myelopathies - a new aetiology. *Trends Neurosci.* 11, pp. 526–532

Roman, G.C., (1994): An epidemic in Cuba of optic neuropathy, sensorineural deafness, peripheral sensory neuropathy and dorsolateral myeloneuropathy. *J. Neurol. Sci.* 127, pp. 11–28

Roman, G. and Caballero, B., (1994): Epidemic optic neuropathy in Cuba: Eye findings. *Arch. Ophthalmol.* 112, pp. 691–699

Roman GC (1995): On politics and health: An epidemic of neurological diseases in Cuba. Ann. Intern. Med; 122:530-33

Rose G (2001): Sick individuals and sick populations. Int. J. Epidemiol; 30: 427 – 432

Sadun, A.A., Martone, J.F., Muci-Mendoza, R., Reyes, L., DuBois, L., Silva, J.C., Roman GC, Spencer PS, Schoenberger BI (1985): Tropical Myeloneuropathies: The hidden endemies. Neurology; 35: 1158-1170

Sadun AA, Martone J F, Reyes L, *et al.* (1994): Optic and peripheral neuropathy in Cuba. *JAMA* ;271:663–4

Sadun AA, Kashima Y, Wurdeman AE, Dao J, Heller K, Sherman J (1994): Morphological findings in the visual system in a case of Leber's hereditary optic neuropathy. Clin Neurosci 2:165-172

Sadun AA, Martone JF. Cuba (1994):: response of medical science to a crisis of optic and peripheral neuropathy. *Int Ophthalmol*;18:373–8

Sadun AA, Martone JF, Muci-Mendoza R, *et al.* (1994): Epidemic optic neuropathy in Cuba: eye findings. *Arch Ophthalmol*;112:691–9

Sadun AA, Popose JS, Madigan MC, et al. (1995): AIDS related optic neuropathy: a histological, virological and ultrastructural study. Grafes Arch Cin Exp Ophthalmol 233:387-398

Sadun AA, Win PH, Ross-Cisneros FN, *et al.* (2000): Leber's hereditary optic neuropathy differentially affects smaller axons in the optic nerve. *Trans Am Ophthalmol Soc*: 8

Santiesteban R, Luis S, et al (1993): Aspectos neuro-oftalmologicos de la neuropatia epidemica en Cuba: Etudio de 602 pacientes. Instituto de Neurologia y neuroqirurgico, la Habana, pp 1-22

Santiesteban R, Lamias-Gonzalez et al (1995): Epidemic neuropathy: proposal and arguments to rename Strachan disease as Strachan and Madan disease. Rev Neurol; 25(148): 1950-6

Schoenberg B, Melton l (1993): Epidemiologic approaches to peripheral neuropathy. In: Dyke PJ, Thomas PK et al, Peripheral Neuropathy, 3rd Edn, WB Saunders, Philadelphia, PA, pp 775-783

Scott, H.H., (1918): An investigation into an outbreak of 'central neuritis'. *Ann. Trop. Med. Parasitol.* **12**, pp. 109–195

Serrano C, Rodriguez M et al (1993): Informe del primerano de neuropatia epidemica en la provincial de Pinar del Rio, Hospital clinicquirurgico "Abel Santana" Pinar del Rio, pp 1-68

Singh G, Lott MT, Wallace DC (1989): A mitochondrial DNA mutations as a cause of Leber's hereditary optic neuropathy. N Engl J Med 320:1300-1305.

Smith JL, Hoyt WF, Susae JO (1973): Ocular fundus in acute Leber optic neuropathy. Arch Ophthamol 90:349-354

Solberg Y, Rosner m and Belkin m (1998): The association between cigarette smoking and ocular diseases. Surv Ophthalmol 42:535-47

Spencer P, Roy D, Ludolph A et al (1986): Lathyrism: Evidence for role of the neuroexcitory aminoacid BOAA. Lancet; 2 (8515): 1066-7

Spillane JD (1947): Nutritional disorders of the nervous system. Edinburgh: E&S Linvinstone

Stone EM, Newman NJ, Miller NR, Johns DR, Lott MT, Wallace DC (1992): Visual recovery in patients with Leber's hereditary optic neuropathy and the 11778 mutation. J Clin Neuro-Ophthalmol 12: 10-14

Strachan H (1888): Malarial multiple peripheral neuritis. *Sajou's Annu.* 1, pp. 139–147

Suga S, Lindsey J (1976): Histopathological observations of presbycusis. Ann. Otol. Rhinol. Laryngol; 85: 167-173

Tenhula WN, Xu SZ, Madigan Mc, Heller k, freeman WR, Sadun AA. (1992): Morphometric comparisons of optic nerve axon loss in acquired immunodeficiency syndrome. Am J Ophthalmol 113:14-20

Teja Perez J, Von Braunmuhl J, Jara Valencia J, Marquez MA. (1992): Cuba's Family Doctor Programme. UNICEF/UNFPA/PAHO/WHO/Ministerio de Salud Publica: Ciudad de La Habana.

The Cuba neuropathy field investigation team. Epidemic optic neuropathy in Cuba: clinical characterization and risk factors. *N Engl J Med* (1995):333:1176–82.

Thomas, P.K., Plant, G.T., Baxter, P., Bates, C. and Santiago-Luis, R., (1995): An epidemic of optic neuropathy and painful sensory neuropathy in Cuba: clinical aspects. *J. Neurol.* 242, pp. 629–638

Tor-Agidbye J, Pamer U, et al (1998): Dietery deficiency of cystine and methionine in rats alters thiol homeostasis required for cyanide detoxification. J Toxicol Enuiron health A; 55: 583-95

Torroni, A., Brown, M.D., Lott, M.T., Newman, N.J., Wallace, D.C., Cuban neuropathy Field Investigation team, (1995): African, Native American and European mitochondrial DNA in Cubans from Pinar del Rio province and implications for the recent epidemic neuropathy in Cuba. Hum. Mutat. 5, 310-317

Toscano A, Harding AE, Cellera C, Vita G, Castagna I, Romeo G, Sweeney MG, Bresolin N, Zeviani M, Messina C (1992): Complex I multiple mitochondrial DNA mutations and biochemical deficiency in Leber's hereditary optic neuropathy. Neurology 42(Suppl 3):266

Trolli G (1938): Paraplegie spastique epidemique, "Konzo" des indigenes du Kwango: In trolli G (ed): Resume des observations reunis, au Kwango, au sujet de deux affections d'origines indeterminee. Brussels, fonds Reine Elisabeth, pp 1-36

Tylleskär, T., Banea, M., Bikangi, N., Fresco, L., Persson, L.A. and Rosling, H., (1991): Epidemiological evidence from Zaire for a dietary etiology of Konzo, an upper motor neuron disease. *Bull. W.H.O.* 69, pp. 581–589

Tylleskär, T., Banea, M., Bikangi, N., Cooke, R.D., Poulter, N.H. and Rosling, H., (1992): Cassava cyanogens and Konzo, an upper motoneuron disease found in Africa. *Lancet* 339, pp. 208–211

Wakakura M, Yokoe J, (1995): Evidence for preserved direct pupillary light response in Leber's hereditary optic neuropathy. Br J Ophthalmol 79: 442-446

Wallace DC (1970): A new manifestation of Leber's disease and a new explanation for its unusual pattern of inheritance. Brain 93 121-132

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Ellas LJ, Nikoskelainen EK (1988): Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427- 1430

Wallace DC. Mitochondrial diseases in man and mouse. *Science* (1999):283:1482–8.

Walters JH (1947): Dietetic deficiency syndromes in Indian soldiers. *Lancet* 252; p. 861

Whitbourne, D., (1947): Nutritional retrobulbar neuritis in children in Jamaica. *Am. J. Ophthalmol.* 30, pp. 169–171

Wilson J (1963): Leber's hereditary optic atrophy-some clinical and etiological considerations. Brain 86:347-362

Winward KE, Hamed LM,, Glaser JS. (1989): The spectrum of optic nerve disease in human immunodeficiency virus infection. Am J Ophthalmol 107: 373-380

Wingerchuck DM, Hogancamp WF, O'Brien PC, Weinshenker BG: The clinical course of Neuromyelitis optica (Devic's Syndrome). Neurology 53:1107-14

Van Senus AHC (1963): Leber's disease in the Netherlands. Doc Ophthalmol 17:1-163

Appendix 1: Epidemic Optic Neuropathy – Clinical examination record

Name_____ Age_____ yrs Sex_____ Clinic_____ Case No _____

Address_____

		Visit 1	Visit 2	Visit 3
Examination Date		_____	_____	_____
Time since disease onset		_____	_____	_____
Ophthalmic Examination				
Corrected Visual Acuity:	Right eye	_____	_____	_____
	Left eye	_____	_____	_____
Colour vision	Right eye	_____	_____	_____
Colour field	Right eye	_____	_____	_____
	Left eye	_____	_____	_____
Amsler	Right eye	_____	_____	_____
	Left eye	_____	_____	_____

Name _____ Case No _____

Ophthalmic Assessment

	Visit 1		Visit 2		Visit 3	
	R	L	R	L	R	L
Pupillary response						
Optic Disc						
Normal						
Blurred margins						
Dilated capillaries						
Temporal pallor						
Temporal pigment accumulation						
Atrophy						
Other _____						

Name_____ Case No _____

		Visit 1		Visit 2		Visit 3	
		R	L	R	L	R	L
Nerve fibre layer	Normal						
	Loss of PMB						
	Hypertrophy						
	Loss of foveal reflex						
	Other_____						
Fundus Photograph							

Name_____ Case No_____

Neurological Examination

	Visit 1		Visit 2		Visit 3	
Tandem walking						
	R	L	R	L	R	L
Vibration sensation at ankles						
Cotton wool sensation in lower limbs						
Pin prick sensation in lower limbs						
Temperature sensation in lower limbs						
Joint position sense						
Knee jerk						
Ankle jerk						
Heel shin ataxia						
Rinné's test						
Audiometry						

Name_____ Case No _____

Neurological questions

	Visit 1		Visit 2		Visit 3	
	R	L	R	L	R	L
Burning sensation in your feet						
Constant						
Intermittent						
Occasional						
Numbness in legs						
Constant						
Intermittent						
Occasional						
Difficulty hearing						
Constant						
Intermittent						

Occasional

Name _____ Case No_____

	Visit 1	Visit 2	Visit 3
Difficulty walking			
Constant			
Intermittent			
Occasional			
Difficulty controlling bladder			
Constant			
Intermittent			
Occasional			
Difficulty in memory			
Constant			
Intermittent			
Occasional			

Name_____ Case No_____

Events at time of visual impairment

Occupation _____Case Industry_____

_____ Spouse _____

_____ Father _____

Pregnant _____

Breastfeeding _____

Eye Pain _____

Burning sensation in your feet

Numbness in legs

Difficulty hearing

Difficulty walking

Difficulty controlling bladder

Difficulty in memory

Weight loss

Did you smoke?

How many cigarettes per day?

Did you drink alcohol?

How much per week?

Did you eat cassava?

How often per week?

Did you take chloroquine?

Time for vision to reach its worst point _____ days _____ weeks _____months

Name _____ Case _____

Lifestyle questions

How often do you eat fish?

How often do you eat meat?

Foods normally eaten (at time of first visit)

Morning	Afternoon	Evening
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Beverages

_____	_____	_____
_____	_____	_____
_____	_____	_____

Blood specimen

Urine specimen

Appendix 2: Instruction Codes for Optic Neuropathy Study Questionnaire

Age:	**in years**
Sex :	M for Males, F for females
Case No:	Serial Number
Time since onset :	In weeks
Visual Acuity:	Snellen chart
Colour Vision: I	Ishihara test
Colour field and Amsler :	1:Normal, 2 central scotoma 3 centrocecal scotoma, 4 other 9 Not done
Pupillary response:	1 Normal 2 slow, 3 RAPD
Optic Disc:	1 Normal, 2 blurred margins, 3 dilated capillaries, 4 temporal pallor, 5 temporal pigmentation 6 atrophy, 7 other
Nerve Fibre Layer:	1 Normal, 2 loss of PMB, 3 hypertrophy, 4 loss of foveal reflex, 5 other
Neurologic Examination:	1 normal, 2 abnormal, 9 not done

Audiometric examination:	1 normal, 2 mild loss, 3 severe loss
neurological symptoms:	1 yes, 2 no
Lifestyle questions:	1 every day, 2 at least once a week, at least once a month, 4 less than once a month
Urine/blood samples	1: taken, 2: Not taken