Pure neuritic leprosy in patients from a high endemic region of Colombia

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Summary

Agua de Dios was a leprosarium for leprosy patients’ obligatory isolation (1872–1961). Its leprosy incidence is the highest in Colombia (1.5–7/10 000). Relapses are common. Government grant of US$ 200 per month subsidy is available to patients with disabilities. Spontaneous consultation with neural symptoms is frequent and simulation to get the subsidy has to be considered. We studied 36 subjects (2007–2009), with ages from 29–78, 19 of them men, with neural symptoms of 6 months to 20 years evolution. All had clinical examination, neural symptoms is frequent and simulation to get the subsidy has to be considered. We studied 36 subjects (2007–2009), with ages from 29–78, 19 of them men, with neural symptoms of 6 months to 20 years evolution. All had clinical examination, bacteriological examination, skin and nerve biopsies, electromyography (EMG), PCR for \( M. leprae \), IgM anti-PGL1, and lepromin A. All but two are household contacts of leprosy patients. Symptoms were hypoaesthesia of the hands and feet, and difficulty using hands with loss of muscular strength. None had skin lesions. Three had thickening of ulnar nerve. Lepromin was positive in all; bacteriology and biopsies were negative in all. The speed and amplitude of neural conduction were altered in 34 patients; two women had normal EMG and were considered to be feigning the disease; 21 were diagnosed as PNL by clinical, epidemiological and EMG findings; five of them had a positive PCR and one, high titers for IgM anti PGL1. Nine other subjects had diabetes and six carpal tunnel syndrome (CTS). Slow progression of disease, the lack of neural enlargement and the neural biopsies without inflammation suggest that...
most of these patients could have spontaneously cured PNL, as happens with other cases of paucibacillary leprosy. Diabetes and CTS are important differential diagnoses of PNL. Patients were treated with MDT and received the state subsidy.

Introduction

Leprosy affects nerves since its outset and is a peripheral neuropathy in which skin and other tissue lesions may occur secondary to neural damage. Pure or primary neuritic leprosy (PNL) only involves peripheral nerve trunks without skin lesions. It is a peripheral neuropathy with hypoesthesia or anesthesia of the innervated area, thickening and tenderness of the affected nerve trunks and muscle weakness. PNL is a cause of progressive degeneration of neural function and is very difficult to diagnose, sometimes remaining unidentified for years.

The most frequently affected nerve trunks are the ulnar (in the medial fossae of the olecranon epicondyle), the radial nerve (in the radial fossae), the common peroneal (lateral popliteal) nerve, the median nerve (at the carpal tunnel), the auricular nerve at the lateral side of the neck, and the posterior tibial behind the medial malleolus, all of them accessible to clinical examination. These anatomical places are superficial and have a lower body temperature between 33°C to 36°C and the affected nerves also cross bony structures or pass through tunnels, places of pressure and trauma, which can act as localising factors.

Patients with PNL can have multibacillary (MB: bacilli are demonstrated in skin or nerves) or paucibacillary (PB) leprosy, but most of them are PB: bacilli are not demonstrated in the skin smear or biopsy; for this reason PNL was named pure neuritic tuberculoid leprosy. The antibodies against the phenolic glicolipid 1 (PGL-1) specific of M. leprae are positive in 21% of the cases, but these antibodies are also demonstrated in 9% of the control group. The Mitsuda test in PNL and PB patients is generally positive, showing the capacity of the host to develop granuloma in response to M. leprae infection, and negative in MB patients.

The symptoms of neural involvement in leprosy begin when the nerve has damage to at least 30% of its fibres. Detection of alterations in the nerve amplitude and speed of conduction, sensory, and motor functions, has been shown to be a sensitive method that can detect the early neural damage. Fifteen per cent or less of the patients with PNL may develop one or two cutaneous lesions, typically of tuberculoid or borderline tuberculoid leprosy phenotype. These lesions may occur at any stage of PNL, but are more frequently seen in the beginning of treatment.

The aim of this work was to study subjects who consulted in the 2007 to 2009 period with suspected symptoms of PNL, using several tools such as clinic, epidemiology, serology, bacilloscopy, histopathology, PCR and EMG. These patients live in Agua de Dios, an endemic town of leprosy in Colombia.

Materials and Methods

THE TOWN AND THE SANATORIUM OF AGUA DE DIOS

The town of Agua de Dios was founded in 1872 as a place for the obligatory isolation of leprosy patients, by a law abolished in 1961. Today it has 13,500 inhabitants, of whom 1,100 are old people with leprosy sequelae; 315 are cared for in lodging-houses by nurses and
physicians as a free service provided by the State. Most of them have a monthly subsidy of US$ 200, provided by law to patients with disabilities due to leprosy. The hospital is a sanatorium with specialised attention given free by nurses and physicians. Because of these facts, some patients are sent there from different parts of the country. In the period 1999–2003, 34 new cases of leprosy were registered in Agua de Dios, 21 of them natives of the town, 68% with bacilli demonstrated in slit skin smears (SSS) or by biopsy (multibacillary, MB), representing a prevalence varying between 23 and 39/10 000 inhabitants. Incidence was 1.5–7/10 000 in the same period, the highest for Colombia; 38 relapses were also detected. A clinical and laboratory study of 2,447 school children of the town, carried out in 2007, discovered four paucibacillary (PB) patients, a case detection rate of 16/10 000, also the highest in Colombia.

Patients. We included all the subjects who consulted the Agua de Dios Hospital for clinical symptoms compatible with PNL from 2007 to 2009.

Clinical examination. A general clinical examination looking for skin patches or infiltration, with emphasis on skin sensitivity and enlargement of nerve trunks affected by leprosy, was performed, looking for nerve thickness and pain as signs of neuritis. The cutaneous sensitivity was explored using dry and alcohol-wet cotton swabs for the tactile and cold sensation and with pins to explore touch and pain. Also, assessment of loss of muscle strength, deformity of fingers and toes, corneal sensitivity and rhinoscopy were performed.

Laboratory tests

Bacteriological index. Slit skin smears (SSS) from ear lobes and two anatomical areas of hypo or anesthesia were stained with Ziehl-Neelsen (ZN) to detect acid fast bacilli, according to the procedures recommended by the Colombian National Program of Leprosy Control.

Skin Biopsies. Skin biopsies of 10 mm long, 5 mm wide and 7 mm deep were taken from the areas with hypo or anesthesia, in order to be sure of the presence of deep cutaneous nerves, the more frequently affected in leprosy. The biopsies were stained with hematoxylin eosin (HE), ZN and with immuno-histochemistry (IHC) for the S100 protein.

Electromyography (EMG). All patients were studied with EMG; the amplitude and speed of sensitivity and motor nerve conduction of the more frequently affected nerves in PNL was tested, except for the auricular nerve. The EMG was performed using the Cadwell Sierra Wave equipment, following standardised protocols.

Nerve biopsies. Biopsies of the sural nerve or the index branch of the radial cutaneous nerve were taken, which were stained with HE, ZN, and with IHC for the S100 protein. Small fragments of these biopsies were included in epoxy resins to make 0.5 µm thick sections that were stained with toluidine blue.

Mitsuda reaction. Lepromin A (0.1 ml) produced by the Schieffelin Leprosy Research Sanatorium, Karigiri, India, was inoculated in the left forearm of all the patients. The reaction was evaluated 21 days after application. A ≥ 4 mm erythematous induration or nodule was considered as positive reaction.

IgM anti phenolic glycolipid I (PGL-1). Titers of IgM anti PGL-1 were detected in serum following published techniques.

PCR for detection of M. leprae DNA. PCR for RLEP1-2 and nested PCR for RLEP3, 4 in nasal swabs, skin smears, and skin and nerve biopsies were performed following established protocols.
One room was used to develop the DNA extraction procedure and another separate room was used to make the PCR reaction. Both procedures were carried out inside class II laminar flow hood, after previous disinfection and UV sterilisation. Barrier pipette tips were used for each step. Ultra-pure DNase/RNase-free distilled water was used for DNA extraction purposes and as a negative and positive DNA control for PCR reaction. Post PCR room was also used to perform the gel electrophoresis for PCR products visualization in 2.5% agarose gel run during 2 h at 80 volts.

First PCR was performed using the LP1 and LP2 primers, and then a nested PCR was carried out using the primers LP3 and LP4 following the protocol for amplification described by Donoghue et al., an amplification band of 99 pb was expected. Samples were tested in duplicate. Sequences of the primers used are:

LP1 5'-TGCATGTCACTGGCCTTGAGG-3'
LP2 5'-CACCGATACCAGCGGCAGAA-3'
LP3 5'-TGAGGTGTCGGCGTGGTC-3'
LP4 5'-CAGAAATGGTGCAAGGGGA-3'

M. leprae strain NHDP-63 (human origin) propagated in armadillos at National Hansen’s Disease Program (NHDP), Baton Rouge, LA was used as the positive control. The infected tissue was processed and M. leprae DNA was extracted at Colorado State University (CSU) in Fort Collins, CO (NIH-NIAID Leprosy Research Support NO1-AI-25469). Negative control included PCR master mix, primers, Taq polymerase, and water instead DNA template. Visualisation of the products was done by agarose gel electrophoresis, ethidium bromide staining and UV transillumination.

Criteria for diagnosis of PNL. These six criteria were initially considered: 1. Clinical symptoms and signs: thickening of peripheral nerve trunks, hypoesthesia or anesthesia of hands and feet, hypotrophy or atrophy of hand muscles and difficulty of hand use, especially in household contacts of leprosy patients. 2. Neuritis or perineural inflammation in nerve or skin biopsies. 3. Demonstration of acid-fast bacilli in skin or nerves. 4. PCR positive for DNA of M. leprae. 5. Positive anti-GLP-1 IgM antibodies. 6. Altered EMG in absence of other entity to explain this alteration. The clinical and epidemiological findings plus the EMG alteration were finally accepted as sufficient criteria to diagnose PNL.

ETHICAL ASPECTS

This project was evaluated by the ethical committees of the Universidad de La Sabana and from de Agua de Dios Sanatorium following the legal dispositions of the resolution No. 008430 of 1993 of the Ministry of Health of Colombia, and was considered of minimal risk. The patients received information about the study and accepted and signed an informed consent.

Results

Patients’ characteristics. Thirty-six subjects living in Agua de Dios spontaneously consulted having hypoesthesia, anesthesia and dysesthesias mainly in hands and arms, with different grades of impairment of the motor function, and similar alterations in legs and feet, without
presence of skin or nasal lesions. None of them had previously received anti-leprosy treatment. Nineteen of 36 (53%) of the patients were male. Their age was in the range 25–78 years, with a mean of 48. All the subjects complained of sensory and motor alterations, with different duration of symptoms, ranging from 6 months to 20 years. Except for two subjects all were household contacts of one or more leprosy patients (blood relatives or spouses).

Nerve thickening (ulnar) was found in only three patients. The bacillary index was negative in all cases. The skin biopsies did not show changes compatible with leprosy in any of the subjects, except that in three of them the nerve fibres showed mild fibrosis, best confirmed by S100 technique which evidenced the presence of abundant collagen around the positive neural fibres.

The biopsies of the sural and the index branch of the radial cutaneous nerves did not show inflammatory changes. The thin sections of nerves stained with toluidine blue showed a demyelination of large fibres and mild endoneural fibrosis in four cases.

EMG readings in 34/36 patients were abnormal, with prolonged latency and slower amplitude and velocity of neural conduction in one or more nerve trunks. Two women with neurological symptoms showed no abnormalities in the EMG, so they were suspected of feigning disease.

The Mitsuda test was positive in all the 36 patients. PCR for *M. leprae* DNA was positive in nerve biopsies of five patients only, and one of them showed high IgM anti PGL-1 positive titers (Table 1).

Twenty-one patients were diagnosed as PNL according to epidemiological, clinical, EMG, and laboratory tests results (Tables 1 and 2). These included clear epidemiologic contact with leprosy patients, anesthesia of hands and feet, reduced muscular strength and EMG changes with lowering of amplitude and sensory conduction velocity of nerve trunks (Table 1). Amongst these 21, only three had thickened nerves, five had PCR positive nerve biopsies, one was PGL-1Ab positive and four showed signs of nerve fibrosis on histology (Table 1). All were treated with MDT for MB leprosy.2

Neuropathy probably had an alternative cause in 13 subjects. Six subjects showed dysesthesic neural symptoms restricted to the upper limbs without neural thickening, and periods of exacerbation and improvement; impaired speed of nerve conduction and amplitude was confined to median nerves. These were diagnosed as carpal tunnel syndrome (CTS),27–29 (Table 2).

Three of the subjects with CTS and other six subjects with dysesthesias without neural thickening had glucose levels higher than 126 mg/dl, and were considered diabetic; further studies for diabetes diagnosis were suggested to these people.30

Discussion

The symptoms of the subjects included in this study were strongly suggestive of PNL because of the epidemiological setting, hypoesthesia/anesthesia, dysesthesias and loss of muscle strength of hands and feet. Only three of them had discreet thickening of the ulnar nerve, but this important sign of PNL is not essential for its diagnosis.4,14 The presence of disability Types 1 or 2 in hands, with digital clawing and thenar/hyphothenar hypotrophy or atrophy are suggestive symptoms of PNL (Table 1).3–5,14,17

The suggestive clinical picture of PNL coupled with the high epidemiological risk of disease18,19 are criteria that should lead to further detailed studies, and if these are not readily
<table>
<thead>
<tr>
<th>No</th>
<th>Age/Sex</th>
<th>Evolution</th>
<th>Symptoms and signs</th>
<th>Leprosy contacts</th>
<th>PCR</th>
<th>Electromyography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69-M</td>
<td>4 y</td>
<td>Hypoesthesia of hands and legs, clawing of 4 and 5 fingers, right hand</td>
<td>Parents and grandparents</td>
<td>Positive</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>2</td>
<td>52-M</td>
<td>6 y</td>
<td>Hypoesthesia in four limbs</td>
<td>Parents and siblings</td>
<td>Positive</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>3</td>
<td>29-M</td>
<td>3 y</td>
<td>Hypoesthesia in hands and arms, loss of muscle strength, thickness of left ulnar nerve</td>
<td>Brother</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>4</td>
<td>35-M</td>
<td>4 y</td>
<td>Hypoesthesia in four limbs</td>
<td>No</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>5</td>
<td>34-M</td>
<td>6 months</td>
<td>Hypoesthesia in hands, forearms and feet. Loss of muscle strength</td>
<td>Mother and sister</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>6</td>
<td>46-M</td>
<td>6 months</td>
<td>Hypoesthesia in arms and forearms</td>
<td>Parents</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>7</td>
<td>49-M</td>
<td>1 y</td>
<td>Hypoesthesia in four limbs</td>
<td>No</td>
<td>Positive</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>8</td>
<td>35-F</td>
<td>4 y</td>
<td>Hypoesthesia in four limbs</td>
<td>Grandparents, mother and uncles</td>
<td>Positive</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>9</td>
<td>50-F</td>
<td>7 y</td>
<td>Hypoesthesia in hands and forearms, loss of muscle strength, thickness of left ulnar nerve</td>
<td>Father</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>10</td>
<td>51-M</td>
<td>10 y</td>
<td>Hypoesthesia in four limbs</td>
<td>Siblings and uncles</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>11</td>
<td>56-M</td>
<td>8 y</td>
<td>Hypoesthesia in hands and elbows, thickening of left ulnar nerve, left hypothenar atrophy, loss of muscle strength</td>
<td>Grandparents and father</td>
<td>Positive</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>12</td>
<td>40-F</td>
<td>3 y</td>
<td>Hypoesthesia in left hand, arm, feet</td>
<td>Grandmother</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>13</td>
<td>64-F</td>
<td>3 y</td>
<td>Hypoesthesia in hands, forearms and feet</td>
<td>Parents, siblings, spouse</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>14</td>
<td>40-F</td>
<td>4 y</td>
<td>Hypoesthesia in hands and forearms</td>
<td>Parents</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>15</td>
<td>50-M</td>
<td>2 y</td>
<td>Hypoesthesia in hands and legs, loss of muscle strength</td>
<td>Wife</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>16</td>
<td>40-M</td>
<td>4 y</td>
<td>Hypoesthesia in hands, forearms and legs, loss of muscle strength</td>
<td>Parents</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>17</td>
<td>78-F</td>
<td>20 y</td>
<td>Hypoesthesia of forearms and legs, anesthesia of hands and feet</td>
<td>Uncles</td>
<td>Negative</td>
<td>SMPN, four limbs</td>
</tr>
<tr>
<td>18</td>
<td>51-F</td>
<td>3 y</td>
<td>Hypoesthesia of hands, loss of muscle strength</td>
<td>Parents, Uncles</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>19</td>
<td>33-M</td>
<td>1 y</td>
<td>Hypoesthesia in four limbs</td>
<td>Parents, grandparents</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
<tr>
<td>20</td>
<td>39-M</td>
<td>1 y</td>
<td>Hypoesthesia of hands and legs</td>
<td>Parents</td>
<td>Negative</td>
<td>SMPN SL</td>
</tr>
<tr>
<td>21</td>
<td>67-F</td>
<td>1 y</td>
<td>Hypoesthesia in hands, forearms and feet</td>
<td>Father</td>
<td>Negative</td>
<td>SMPN four limbs</td>
</tr>
</tbody>
</table>

available, patients should be managed and treated as PNL. In these studies, changes in amplitude and nerve conduction speed were a key finding to confirm the presence of nerve damage. Based on these clinical, epidemiological and EMG criteria, 21 patients were diagnosed as having PNL. We did not find evidence of other causes of neural damage, such as hereditary sensory-motor neuropathy, exposure to toxic substances, alcohol ingestion, paraneoplastic syndromes, or consumption of drugs such as thalidomide or sulfone. HIV tests were not done.

Surprisingly, histopathological changes in neural biopsies were not found, possibly due to spontaneous resolution of the disease or to failures in nerve sampling (changes may be patchy). A biopsy of an affected mixed nerve trunk according to EMG confirmed alteration was not considered. Histopathology is a very useful tool to diagnose PNL if it shows diagnostic intraneural epithelioid granulomas; however, in a report it was positive only in 15% of the cases.

The PCR technique was done with proper controls, following the published recommendations. It was positive only in nerve tissue from five patients and negative in skin biopsies and in skin and nasal swabs. This finding could mean a limited neural disease, as supported by other findings such as negative bacteriological index, positive Mitsuda test and absence of other signs and symptoms. One PNL patient showed PCR positive and IgM anti PGL1 positive titers, a similar immunological response to that of a MB patient. The other 20 patients did not show positive titers for PGL1, a fact that can be attributed to the type of immune response typical of PB patients.

It is possible also, that some of these patients with PNL may have cured spontaneously, through the immune host response, as happens with other forms of PB leprosy, a hypothesis which deserves further work.

As the Colombian National Leprosy Program provides by law a subsidy of US$ 200 per month as economical support for leprosy patients, especially those with disabilities, it is common to find inhabitants of this region who pretend to have leprosy to get the subsidy. Due to this fact, and to deliver the proper treatment, complementary studies to confirm leprosy have to be conducted to establish the diagnosis. EMG is the most precise test because it detects early neural damage. It also allows studying all peripheral nerve trunks at a relative low cost.

Only two women of this study showed a normal EMG; one of them had clawing of the fourth and fifth fingers of her left hand and complained of severe pain on palpation of the ulnar nerve, which was not thick. The frequency of abnormalities found in the EMG tests help to rule out a hypothesis in vogue among the local medical personnel that suggests that most people with symptoms suggestive of PNL simulate them to get the government subsidy.

Table 2. Final Diagnostic of the studied patients

<table>
<thead>
<tr>
<th></th>
<th>diagnosis</th>
<th>count (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PNL</td>
<td>21 (58.4%)</td>
</tr>
<tr>
<td>2</td>
<td>Diabetes mellitus</td>
<td>7 (19.5%)</td>
</tr>
<tr>
<td>3</td>
<td>Carpal tunnel syndrome</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td>4</td>
<td>Carpal tunnel syndrome plus diabetes</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td>5</td>
<td>Feigned symptoms</td>
<td>2 (5.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>36 (100%)</td>
<td></td>
</tr>
</tbody>
</table>
The EMG allowed us to detect another group of patients with symptoms restricted to the upper limbs, without neural thickening, and with periods of exacerbation and improvement and in which there were only impaired speed of nerve conduction and amplitude of median nerve, so these six patients were diagnosed as having CTS, an entity that can be confused with neural leprosy. Thickening of the median nerve suggests leprosy, in contrast to CTS in which the median nerve is compressed and thinned.

Ten patients (including three of those with CTS) showed levels of glycaemia higher than 126 mg/dl, so they were considered diabetic and excluded as PNL patients (Table 2).

We believe that in endemic areas for leprosy, clinical findings of persistent skin hypothesia on anesthesia, difficulties in hand gripping, with or without neural trunk thickening, should indicate the possibility of PNL, even more so if suspects have relatives with confirmed leprosy. Laboratory tests are indicated, especially EMG. Even if bacteriology, neural biopsy, anti-phenolic glycolipid antibodies and PCR for leprosy bacilli are negative, the suspect should be managed as PNL. The possibility of spontaneous cure of PNL should be considered and deserves further research.

These subjects were self-referred suspects who form an unknown proportion of the affected population. The background created by the government subsidy is a factor causing confusion. We were unable to carry out further blood tests to rule out nutritional, toxic, auto-immune or other infective causes of neuropathy, but we feel they are highly unlikely in the region, where cases are detected only in Agua de Dios and not in numerous nearby towns. We did not have data on follow up of the 21 subjects to comment on the extent of recovery or deterioration of nerve function impairment after receiving MDT. All of these 21 patients diagnosed with PNL received the government subsidy.

In summary, 21 of 36 subjects initially suspected of PNL were confirmed with this diagnosis, based mainly on epidemiological, clinical findings and on changes of amplitude and nerve conduction on EMG. Five of them had positive PCR for \textit{M. leprae} DNA in the sural nerve biopsy, a technique that is diagnostic for leprosy and was reported positive in 47\% of patients with PNL in a study from Brazil.

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**Conflict of interest.** The authors declare no conflicts of interest in the preparation or publication of this work.

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