

Study of the profile of the neurotrophin BDNF in new leprosy cases before, during and after multidrug therapy

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in the survival of neurons and growth and differentiation of dendrites and axons. The purpose of the present study was to evaluate plasma levels of BDNF of leprosy patients at different stages of multidrug therapy (MDT) in comparison with non-infected individuals. Plasma levels of BDNF were measured by ELISA in 30 healthy controls and 37 leprosy patients at diagnosis, during and after MDT. Plasma levels of BDNF tended to be higher in control subjects in comparison with leprosy patients, but this difference does not reach statistical significance. Interestingly, BDNF levels changed following MDT, achieving statistical difference only at the 2nd dose of MDT. These results indicate that BDNF may not be a surrogate marker of leprosy infection and/or related neuropathy. Further research is needed to investigate the meaning of BDNF level changes following leprosy treatment.

Key words: leprosy, BDNF, peripheral neuropathy, biomarker, follow-up.

Estudo do perfil da neurotrofina BDNF em casos novos de hanseníase antes, durante e após poliquimioterapia

RESUMO

O fator neurotrófico derivado do cérebro (BDNF) é uma neurotrofina envolvida na sobrevivência neuronal e no crescimento e diferenciação dos dendritos e axônios. O objetivo do presente estudo foi avaliar os níveis plasmáticos do BDNF de pacientes com hanseníase em diferentes fases da poliquimioterapia (PQT), em comparação com indivíduos não-infectados. Os níveis plasmáticos do BDNF foram mensurados pelo teste ELISA em 30 controles sadios e 37 pacientes com hanseníase no momento do diagnóstico, durante e após PQT. Os níveis plasmáticos do BDNF mostraram-se maiores nos indivíduos controles em comparação com os pacientes com hanseníase, mas não houve diferença estatisticamente significativa. Curiosamente, os níveis de BDNF modificaram-se com o tratamento, mostrando diferença estatística apenas na segunda dose de PQT. Esses resultados indicam que o BDNF pode não ser um marcador de infecção na hanseníase e/ou neuropatias relacionadas. Novas pesquisas são necessárias para investigar o significado das alterações nos níveis de BDNF ao longo do tratamento da hanseníase.

Palavras-chave: hanseníase, BDNF, neuropatia periférica, biomarcador, seguimento.

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Leprosy is a contagious, granulomatous and chronic infectious disease caused by *Mycobacterium leprae*, an obligate intracellular bacillus that affects mainly the skin and peripheral nerves¹⁻³. It can be better understood when regarded as an association of two diseases: first, a chronic infection that induces a cell-mediated immune response; second, a peripheral neuropathy which is triggered by infection and followed by a series of immunological events, whose evolution, sequels and disabilities often last many years, even after infection has ceased⁴⁻⁷.

Leprosy still remains the commonest cause of peripheral neuropathy in developing countries⁸. A recent important contribution to the understanding of leprosy pathogenesis has been the elucidation of the molecular basis for the entry of *M. leprae* into the Schwann cells and the peripheral nerve. The demonstration of rapid demyelination and axonal atrophy induced by *M. leprae* provides a model for elucidating the molecular events of early nerve degeneration which might be common to neurodegenerative diseases of both infectious and unknown etiology⁹⁻¹². In addition, *M. leprae* may directly induce Schwann cells to secrete metalloproteinases independent of the extent of inflammation in leprous neuropathy¹³.

Neurotrophins are soluble polypeptide molecules that act on neurons of the central and peripheral nervous system (PNS), being involved in their survival and differentiation. These molecules also help stimulate and control neurogenesis, the brain-derived neurotrophic factor (BDNF) being one of the most active^{14,15}.

According to several reports on neurodegenerative disorders, neurotrophin levels generally differ from those found in healthy subjects. For instance, several studies have shown altered circulating levels of BDNF in Alzheimer's disease, Parkinson's disease and schizophrenia^{16,17}. Gagliardi¹⁸ studied diabetic peripheral neuropathy and reported that the patients' capacity to maintain the normal structure and function of their nerves may depend on the expression and effectiveness of these neurotrophins.

It is known that BDNF is synthesized by a wide variety of cells and tissues, including Schwann cells, and has different modulating functions in the PNS myelination program, with highly specific regulated mechanisms of action¹⁹. The role of neurotrophins in peripheral neuropathies, including leprosy, still remains open²⁰. Circulating levels of BDNF of leprosy patients have not been assessed up to now. Thus, the purpose of the present study was to evaluate plasma levels of BDNF of leprosy patients in comparison with non-infected individuals and at different stages of multidrug therapy (MDT), assessing its potential as a biomarker of nerve injury.

METHOD

This is an exploratory, descriptive, longitudinal and analytical study, including 37 new cases of leprosy mon-

itored along standard multidrug therapy (MDT), (median age, years [range]: 48 [26.0-77]; M/F: 20/17). The research was carried out at the Dermatology Ambulatory of the Eduardo de Menezes Hospital, Minas Gerais State Hospital Foundation (FHEMIG), in Belo Horizonte, Brazil, from May 2006 to December 2007.

Data collection started after the project was approved by the Committee of Ethics in Research of two hospitals: the Eduardo de Menezes Hospital and the Santa Casa de Misericórdia of Belo Horizonte. All the subjects gave their informed consent to participate. Diagnosis and classification of leprosy were based on clinical assessment by taking a detailed history, careful medical and dermatological examination and detection of acid-fast bacilli in skin slit smears by an experienced technician. All leprosy patients but four were not classified as multibacillary according to the operational criteria of the World Health Organization²¹. Only patients who showed a positive response to treatment with complete clinical remission were included in this study, preventing any confounding effect due to lack of response to treatment. No patient developed leprosy reactions in the follow-up of treatment.

Non-infected (NI) individuals comprised age and gender-matched individuals (median age, years [range]: 46.5 [26-75]; sex M/F: 17/13). NI group was composed by healthy members of the community.

In leprosy patients, blood collection occurred four times (pre-treatment; 2nd dose of MDT; 6th dose and post-MDT); in healthy controls it occurred once. The number of patients submitted to peripheral blood collection at intermediate doses (i.e. 2nd and 6th doses) was lower than the number of samples at pre and post-treatment due to non-attendance of some patients. After blood centrifugation, plasma was collected and stored until analysis. Plasma levels of BDNF levels were measured by sandwich ELISA following the protocol provided by the manufacturer (R&D Systems, Minneapolis, USA). Concentration of BDNF was expressed in pg/ml.

For data analyses, the following methods were used: measures of central tendency and variability; the Mann-Whitney statistical tests for comparing BDNF levels in leprosy cases in pre-treatment and healthy controls; Wilcoxon test for comparing molecule levels in leprosy patients in pre-MDT and other treatment stages; and the generalized linear model (GLM) for repeated measures with four factors (F test), for comparing the kinetics of BDNF levels along treatment. GLM was restricted due to the small size of the sample, as only seven patients had their BDNF levels measured at all time points (pre-MDT, 2nd dose, 6th dose and post-MDT). In addition, a dispersion graph was used to assess the association between the BDNF levels in leprosy patients in the pre- and post-MDT and the number of affected nerves. The significance level adopted was 5%.

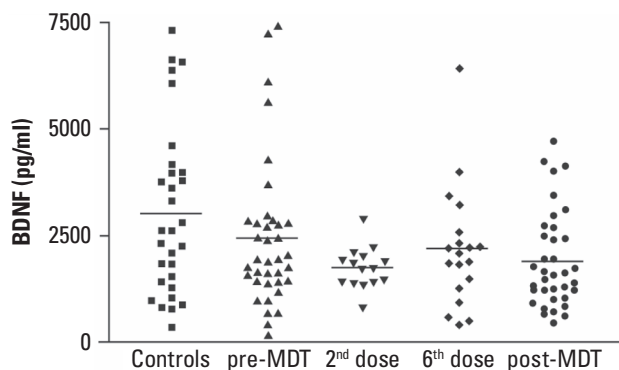


Fig 1. Dispersion of the BDNF measurements for healthy controls and treated subjects in each treatment phase.

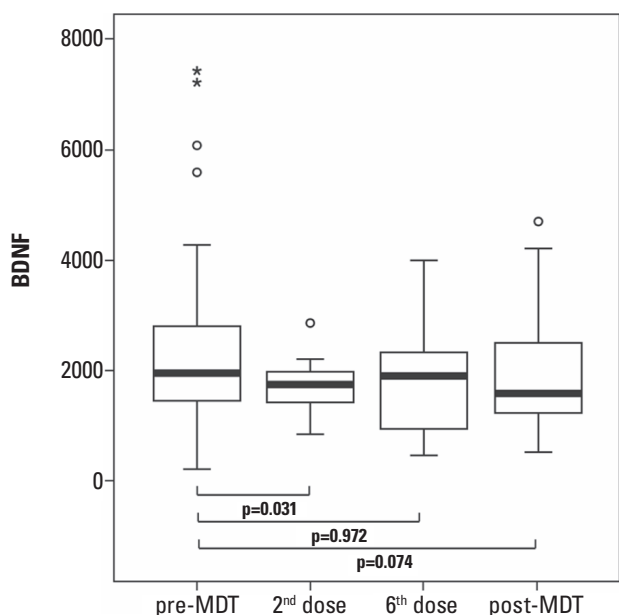


Fig 2. P-values for the BDNF measurements in the comparison of pre-treatment and the other phases.

RESULTS

Most patients were male, with a mean age of 50 years, and had more than five skin lesions and more than one nerve affected. Most patients were classified as multi-bacillary (MB), while 37.8% were positive at bacilloscopy.

BDNF levels were decreased in leprosy patients before treatment in comparison with the healthy controls, but this difference did not reach statistical difference (Fig 1).

With regard to BDNF levels in leprosy patients in pre-treatment and other periods (2nd dose, 6th dose and post-MDT), a significant difference was found only between pre-treatment and 2nd dose (Fig 2). The F-test, involving a sample of 7 leprosy patients who had their blood collected all time points, showed no significant difference in BDNF levels along the study, but graphic analysis re-

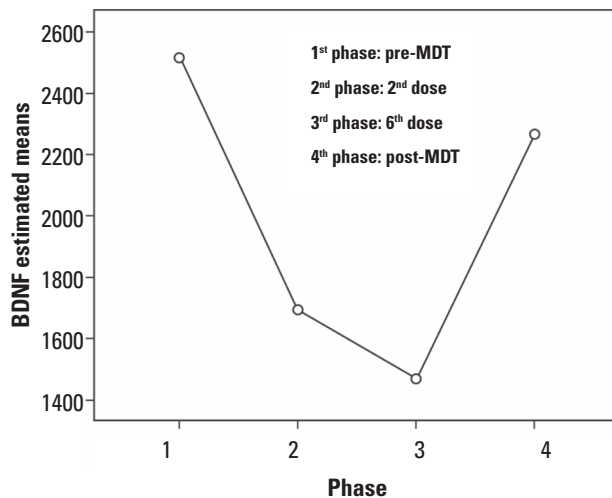


Fig 3. Graphic representation of the BDNF means at the four-phase study of seven leprosy cases at the FHEMIG Eduardo de Menezes Hospital, Sanitary Dermatology Reference Service.

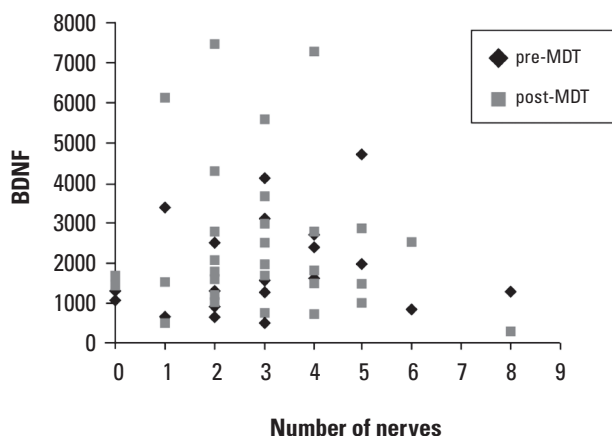


Fig 4. Relationship between the BDNF levels in the pre- and post-treatment cases and the number of nerves affected.

vealed reduced BDNF levels after the beginning of treatment, with a tendency to recover with time (Fig 3). In addition, dispersion graph showed no significant association between BDNF levels and the number of affected nerves (Fig 4).

DISCUSSION

Although leprosy is known as a disease that damages peripheral nerves, little is known about the exact mechanism of the resulting neuropathy. As a consequence, much effort has been made to investigate possible neural markers of the disease²⁰⁻²³.

BDNF is expressed by numerous cells and tissues, including Schwann cells, playing a relevant role in neural development and plasticity. It is known that most indi-

viduals can maintain constant BDNF levels when they become adults²⁴. Therefore nerve damage following leprosy infection combined with other factors, such as the stress related to the stigma surrounding the disease, could result in decreased levels of BDNF in patients. Nevertheless, the present study showed no significant difference in BDNF levels between leprosy patients and healthy controls, giving no support to this hypothesis.

The comparison between BDNF pre-treatment levels and the other time points showed a significant difference only between pre-treatment and MDT 2nd dose levels. The reduction of BDNF levels following the onset of MDT may result from the massive death of *M. leprae* bacilli and the associated Schwann cells lesion. When the kinetic of BDNF levels was analyzed along different phases of MDT in a subgroup of patients, no significant difference was found, but BDNF also decreased after the beginning of treatment. Interestingly, following this decline, BDNF level tends to increase with time, showing a tendency to recover its baseline level after MDT completion. This result must be seen as preliminary as only few patients had their samples collected at all time points. Further studies involving more patients and with a longer follow-up are necessary to confirm this.

The subclinical loss of nerve function, both sensory and mixed, is largely described in the literature, but there is a great need of search for reliable markers in early diagnosis and follow-up²⁵⁻²⁸. Previous studies have investigated the value of different approaches such as systematic clinical evaluation of nerve function, electrophysiology and immunohistochemistry²⁵⁻²⁷. Clinical examination using the Semmes-Weinstein monofilament shows great sensitivity and specificity in nerve monitoring²⁷.

The neuromodulatory action of BDNF has been proposed in models of inflammation, peripheral nerve injury and neuropathic pain^{29,30}. No statistically significant difference was found to confirm the possible association between the BDNF levels and the number of nerves affected. This can be attributed to subjectivity (or examiner-dependency) in the criterion for the evaluation of nerve damage, which has already been excluded from the classification of clinical forms of Hansen's disease.

In addition, factors with which it cannot interfere, such as genetic, nutritional status and likely the parallel use of other drugs may constitute limiting factors to infer conclusions about the results presented.

There are some other limitations in the present study that should be mentioned. One of them is the classification of patients based solely on clinical parameters. The classification of patients according to the Ridley-Jopling criteria was not possible because biopsy specimens are not routinely obtained in most regions where the disease is endemic. A careful clinical observation, by monitoring

disease progression during treatment, often allows for the correction of errors in the initial classification, even when it is backed by laboratory data patterns considered, such as skin biopsy²⁸. Another one is the size sample. These nets need to be addressed in further studies.

In conclusion, our data suggest that BDNF may not be useful as a biomarker of neuropathy associated with leprosy. This study was the pioneer of a line of research involving neurotrophins in leprosy, which claims to evaluate their expression and function in leprotic neuritis and in reactional episodes, in order to confirm their presence as a predictive marker and a parameter for monitoring the patients' neural affection in future clinical trials. Moreover, the understanding of the biology of neurotrophins can contribute to the possible adoption of new therapeutic approaches.

REFERENCES

- Munk ME, Anding P, Schettini AP, Cunha MG, Kaufmann SH. Soluble tumor necrosis factor alpha receptors in sera from leprosy patients. *Infect Immun* 1999;67:423-425.
- Barreto JA, Belone AFF, Fleury RN, Soares CT, Lauris JRP. Manifestações de padrão tuberculóide reacional na hanseníase dimorfa: estudo histoquímico e imuno-histoquímico comparativo, em biópsias cutâneas, entre reações tipo 1 ocorridas antes e durante a poliquimioterapia. *An Bras Dermatol* 2005;80:68-74.
- Pardillo EF, Fajardo TT, Abalos RM, Scollard D, Gelber RH. Methods for the classification of leprosy for treatment purposes. *Clin Infect Dis* 2007;44:1096-1099.
- Mendonça VA, Costa RD, Brito-Melo GE, Antunes CMF, Teixeira, AL. Imunologia da hanseníase. *An Bras Dermatol* 2008;83:343-350.
- Pimentel MIF, Nery JAC, Borges E, Gonçalves RR, Sarno EM. O exame neurológico inicial na hanseníase multibacilar: correlação entre a presença de nervos afetados com incapacidades presentes no diagnóstico e com a ocorrência de neurites francas. *An Bras Dermatol* 2003;78:561-568.
- Rea TH, Modlin RL. Hanseníase. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IL, Austen KF (Eds.). *Tratado de dermatologia*. Rio de Janeiro: Revinter Ltda, 2005:56-85.
- Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. *Clin Microbiol Rev* 2006;19:338-381.
- Haimanot RT, Melaku Z. Leprosy. *Curr Opin Neurol* 2000;13:317-322.
- Shetty VP, Shetty KT, Save MP, Antia NH. *M. leprae* induced alteration in the neurofilament phosphorylation leads to demyelination in leprosy nerves: a hypothesis. *Ind J Lep* 1999;7:121-135.
- Rambukkana A, Yamada H, Zanazzi G, et al. Role of dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. *Science* 1998;282:2076-2079.
- Save MP, Shetty VP. A critique on commentary 'How *Mycobacterium leprae* infects peripheral nerves' by Freedman et al. *Lepr Rev* 2001;72:102-105.
- Arévalo JC, Wu SH. Neurotrophin signaling: many exciting surprises. *Cell Mol Life Sci* 2006;63:1523-1537.
- Oliveira AL, Gomes Antunes SL, Teles RM, et al. Schwann cells producing matrix metalloproteinases under *mycobacterium leprae* stimulation may play a role in the outcome of leprosy neuropathy. *J Neuropathol Exp Neurol* 2010;69:27-39.
- Heumann R, Korsching S, Bandtlow C, Thoenen H. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. *J Cell Biol* 1987;104:1623-1631.
- Meyer G, Wahle P, Castaneyra-Perdomo A, Ferres-Torres. Morphology of neurons in the white matter of the adult human neocortex. *Exp Brain Res* 1992;88:204-212.
- Reis HJ, Nicolato R, Barbosa IG, Prado PHT, Romano-Silva MA, Teixeira AL. Increased serum levels of brain-derived neurotrophic factor in chronic institutionalized patients with schizophrenia. *Neurosci Lett* 2008;445:18-22.
- Scalzo P, Kümmer A, Bretas TL, Cardoso F, Teixeira, AL. Serum levels of brain-

- derived neurotrophic factor correlate with motor impairment in Parkinson's disease. *J Neurol* 2010; 257: 540-545.
18. Gagliardi ART. Peripheral diabetic neuropathy. *J Vasc Br* 2003;2:67-74.
 19. Chan JR, Cosgaya JM, Wu YJ, Shooter EM. Neurotrophins are key mediators of the myelination program in the peripheral nervous system. *Proc Natl Acad Sci USA* 2001;98:661-668.
 20. Facer P, Mann D, Mathur R, et al. Do nerve growth factor-related mechanisms contribute to loss of cutaneous nociception in leprosy? *Pain* 2008;85: 231-238.
 21. World Health Organization (WHO). Guide to eliminate leprosy as a public health problem. Geneva: World Health Organization, 1995: 115-122.
 22. Cunha MGS. Episódios reacionais e relação com recidiva em doentes com hanseníase multibacilar tratados com diferentes esquemas terapêuticos. Tese de Doutorado. Faculdade de Medicina da Universidade de São Paulo: Ribeirão Preto; 2001.
 23. Faber WR, Iyer AM, Fajardo TT, et al. Serial measurement of serum cytokines, cytokine receptors and neopterin in leprosy patients with reversal reactions. *Lepr Rev* 2004;75:274-281.
 24. Peng S, Wu J, Mufson EJ, Margaret F. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* 2005;93:1412-1421.
 25. Little D, Kanolkar-Young S, Coulthart A, Suneetha S, Lockwood DNJ. Immunohistochemical analysis of cellular infiltrate and gamma interferon, interleukin-12, and inducible nitric oxide synthase expression in leprosy type 1 (Reversal). Reactions before and during prednisolone treatment. *Infect Immun* 2001;69:3413-3417.
 26. Garbino JA, Virmond, MCL, Ura S, Salgado MH, Naafs B. A randomized clinical trial of oral steroids for ulnar neuropathy in type 1 and type 2 leprosy reactions. *Arq Neuropsiquiatr* 2008; 66:861-867.
 27. Marciano LHSC, Garbino JA. Comparação de técnicas de monitoração da neuropatia hanseniana: teste de sensibilidade e estudo de condução nervosa. *Hansen Int* 1994; 19:5-10.
 28. Barreto JA, Carvalho CV, Cury Filho M, Nogueira MES, Soares CT. Hanseníase multibacilar com baciloscopia dos esfregaços negativa: a importância de se avaliar todos os critérios antes de se definir a forma clínica. *Hansen Int* 2007;32:75-79.
 29. Pezet S, Spyropoulos A, Williams RJ, McMahon SB. Activity-dependent phosphorylation of Akt/PKB in adult DRG neurons. *Eur J Neurosci* 2005; 21:1785-1797.
 30. Zhao LY, Ye TH, Zhang YZ, Zhao H. Combination of morphine with lowdose naloxone for intravenous patient-controlled analgesia. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2005;27:228-231.