Original Article

Anti-ceramide antibodies in leprosy: marker for nerve damage?

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Abstract

Background: Leprosy is a chronic infectious disease primarily affecting the peripheral nervous system and skin. Multibacillary leprosy is associated with nerve damage which could contribute to myelin alteration. As ceramide is a constituent of myelin sheath, the present study aimed to compare anti-ceramide antibody titre in paucibacillary and multibacillary leprosy patients with controls.

Methodology: Serum levels of anti-ceramide antibody were measured using enzyme-linked immunosorbent assays (ELISA) in 50 leprosy patients (25 paucibacillary and 25 multibacillary) and 25 healthy controls. Results were reported in OD units as mean ± SD and analyzed by Chi square test (significance at $p < 0.05$).

Results: Patients suffering from multibacillary leprosy had significantly higher anti-ceramide antibody serum levels compared to paucibacillary leprosy patients and healthy controls ($p < 0.005$).

Conclusions: Since nerve damage is the most debilitating effect of leprosy, the search for a serum marker for assessing nerve damage is required in countries where leprosy is still widespread. In multibacillary leprosy patients, the role of anti-ceramide antibody as a marker for nerve damage should be explored.

Key words: antibody, multibacillary, paucibacillary, ceramide, nerve

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Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*). It is primarily a disease of the peripheral nervous system and skin. Though the global burden of the disease has decreased tremendously since the introduction of multidrug therapy, nearly half a million cases are estimated to remain worldwide, mainly in the Asian and African subcontinents [1]. The role of the immune system in the pathology of leprous neuritis has always been of interest to immunologists. *M. leprae* is known to preferably reside in Schwann cells, signifying the role of immune system reactivity in nerve damage. Early detection of neuritis can lead to the prevention of nerve damage in leprosy. Antibodies to neuronal glycolipids such as galactocerebroside [2,3], ceramide [2] and asialo GM1 of myelin lead to demyelination causing loss of nerve function. These antibodies have potential diagnostic value in predicting leprous nerve damage [4].

The present study was devised to estimate the anti-ceramide antibody titre in sera of leprosy patients. Since ceramide is a glycosphingolipid that is expressed as a surface determinant of myelin, antibodies to ceramide or related neural components of the myelin sheath may be associated with nerve damage. Though leprosy cases are on the decline globally, they are still prevalent in India. The present study intends to explore whether anti-ceramide antibodies can serve as a reliable marker in the assessment of nerve damage in leprosy patients.

Materials and methods

The study was conducted in the Department of Biochemistry, Maulana Azad Medical College, and associated Lok Nayak and GB Pant Hospitals, New Delhi, and approved by the institutional Ethics Committee. Fifty patients (25 each paucibacillary and multibacillary leprosy) were included in the study, along with 25 age- and sex-matched healthy individual controls. Informed and written consent was obtained from all leprosy patients and controls. Detailed medical histories were taken and clinical examinations were performed. Histopathological report sof skin biopsies was used for diagnosing paucibacillary (PB) and multibacillary (MB) leprosy cases. Leprosy patients were classified broadly as PB

or MB according to World Health Organization (WHO) guidelines for the treatment purposes without taking into account the size and extent of lesions or the number of nerves involved. Venous blood

samples (5 ml each) were collected from both leprosy patients and healthy controls under sterile conditions. Leprosy patients on drug therapy, immunosuppressive therapy such as corticosteroids, regular analgesics and/or with a history of inflammatory, autoimmune disease or any other systemic illness were excluded from the study. Anti-ceramide antibody titre was estimated by indirect ELISA. Reagents such as ceramide (galactosylceramide), BSA (bovine serum albumin), conjugate (goat anti-human IgM HRPO), phosphate buffer saline (0.001M, pH 7.2), citrate phosphate buffer (pH 5.0), methanol, 30% H_2O_2, orthophenylene diamine (OPD) and HCl were provided by Sigma. A microtitre ELISA plate was obtained by Maxisorp, NUNC, Denmark.

Statistical analysis

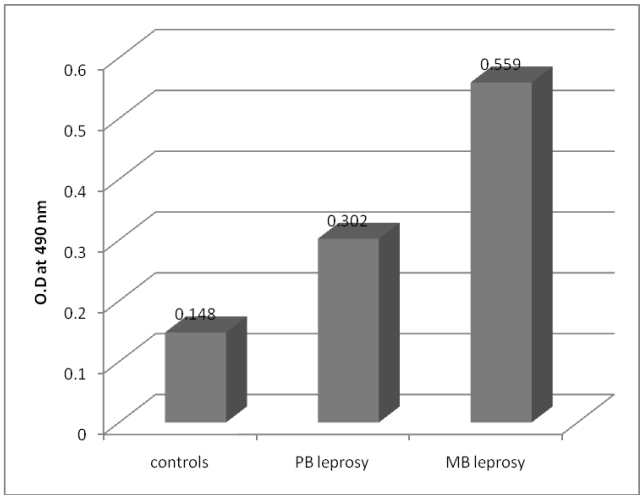
Results were reported as mean ± SD and data was analyzed statistically by Chi square test, with the level of significance set at $p < 0.05$. Statistical analysis was performed using SPSS windows version 12.0 software (SPSS Inc., Chicago, Illinois).

Results

In the present study, anti-ceramide antibody titre assayed from MB and PB leprosy patients was compared with that of healthy controls (Figure 1). Higher levels of serum anti-ceramide antibody titre was observed in 24 (96%) out of 25 cases of MB leprosy and in 15 (60%) out of 25 cases of PB leprosy (Table 1). The mean and standard deviation of optical density (OD) measured at 490 nm was 0.148 ± 0.084 OD units in controls, 0.302 ± 0.172 OD units in PB leprosy patients, and 0.559 ± 0.396 OD units in MB leprosy patients (Table 2). The serum anti-ceramide antibody titre was found to be significantly higher in leprosy patients (both PB and MB) as compared to control subjects. Among the leprosy patients, it was observed to be significantly higher in patients suffering from MB leprosy ($p < 0.005$).

The ROC curve for anti-ceramide antibody titre in MB leprosy patients versus controls shows the area under the curve to be 0.984 ($p < 0.005$) (Figure 2). The analysis showed that at a cut-off value of 0.259 OD units, sensitivity was 100% and specificity was 96%. For PB leprosy, ROC curves show that the area under the curve was 0.742 OD units, and, at a cut-off of 0.264 OD units, sensitivity was 100% and specificity was 52% (figure3).

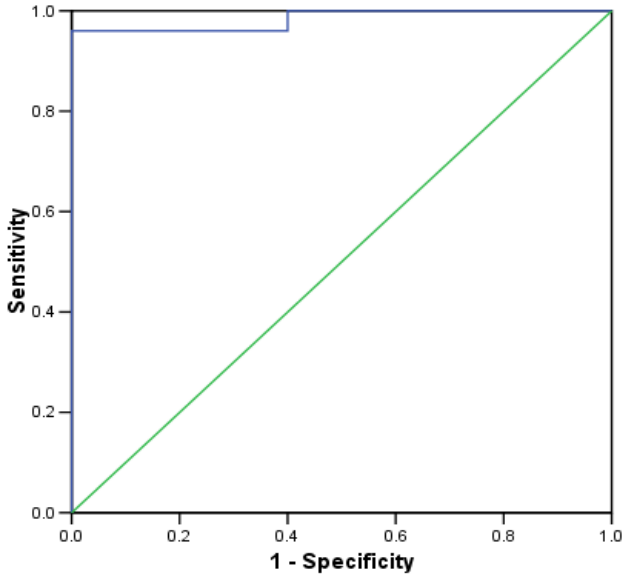
Figure 1. Anti-ceramide antibody titre in study subjects



Discussion

The present study investigated the presence of antibodies to ceramide in leprosy along with its association with the occurrence of nerve damage. Anti-ceramide IgM antibody titre was significantly higher in MB leprosy patients in comparison to both controls and PB leprosy patients. In this study, in which ceramide was considered as a marker of nerve sheath damage, results indicate that *M. leprae* infection is a major factor in inducing anti-neural antibody response.

Figure 2. ROC curve for serum anti-ceramide antibody titre in multibacillary leprosy patients versus controls



Area under the curve 0.984
At cutoff, 0.259 sensitivity is 100% and specificity is 96%

Table 1. Association of anti-ceramide antibody titre between paucibacillary and multibacillary leprosy

		ANTI-CERAMIDE ANTIBODY TITRE		
S. No	**Types of Cases**	**Normal**	**High**	**Total**
1.	**PB (n = 25)**	10 (40%)	15 (60%)	25 (100%)
2.	**MB (n = 25)**	1 (4%)	24 (96%)	25 (100%)
	Total (n = 50)	11 (22%)	39 (78%)	50 (100%)

In the present study, serum anti-ceramide antibody titre of healthy controls ranged between 0.010 and 0.239 OD units. Serum anti-ceramide antibody titre was observed to be significantly higher in MB leprosy patients as compared to PB leprosy patients.

The ROC curve of anti-ceramide antibody titre in MB leprosy patients versus controls shows the area under the curve to be 0.984 ($p < 0.005$) which is highly significant. Here, cut-off values of < 0.259 OD units showed sensitivity to be 100% and specificity to be 96%. If the cut-off value is lowered to 0.253, which would be closer to PB patients, the sensitivity is reduced to 96% though specificity remains the same, suggesting that the cut-off of < 0.259 OD units can be accurately applied as a marker to segregate the MB patients from controls. In the case of PB leprosy, ROC curves show an area under the curve of 0.742 OD units, and, at a cut-off of 0.253 OD units, sensitivity was 96% and specificity was very low at 52%. If the cut-off value is raised to 0.264, sensitivity increases to 100% even though there is no change in specificity. Hence, anti-ceramide antibody titre cannot be used as marker to differentiate between PB and controls.

Antibodies to neural glycolipids have been detected in leprosy patients. Ceramides are found in high concentration within the cell membranes. Antibodies against ceramide, which are prevalent on nerve cells, have been studied in patients with different types of leprosy. Some previous studies have documented associations between antineural antibodies and nerve damage in leprosy. This relationship can be detected in sera of leprosy patients using enzyme-linked immunosorbent assays [5]. No significant difference was found in the prevalence of antineural antibodies between PB and MB leprosy patients, but there was a strong association between antineural antibodies and the degree of nerve involvement, such as extensiveness of anesthesia and enlarged nerves at the time of diagnosis. Two different groups, Vemuri *et al.* and Narayan *et al*., observed higher levels of anti-ceramide antibody titre as compared to the titre of antigalactocerebroside antibodies in MB leprosy patients as compared to PB leprosy patients [2,6]. These findings are in accordance with the results of the present study.

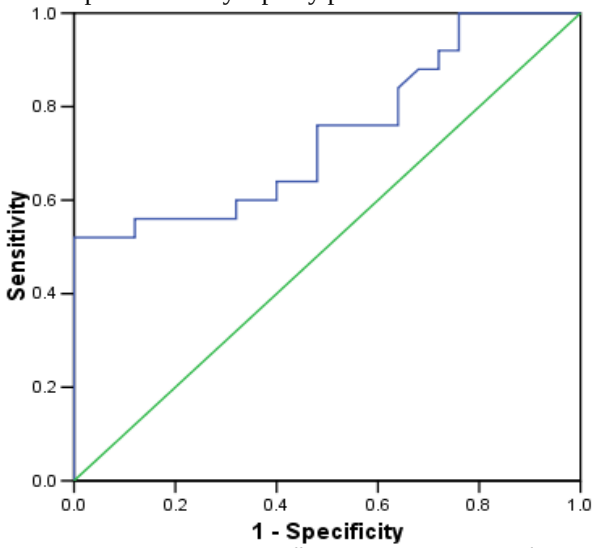
As nerve damage is more common in MB leprosy patients, anticeramide antibodies can serve as a marker for nerve damage by showing the extent of nerve damage, allowing for better management [6,7]. Autoantibodies to cerebroside sulphate might have a role in immune pathology of multibacillary patients. It is suggested that anti-sulphatide IgM is elevated in leprosy patients in relation to the bacterial load and anti-sulphatide IgM, and are consistent with the deposition of serum antibodies [7]. Repeated alpha galactosyl ceramide injections affect the activation of natural killer T cells (NKT) in tumour cells in mice [8, 9, 10]. In multibacillary leprosy patients, both IL-4 and anti-ceramide antibody titres increase. It can be concluded that NKT cells produce IL-4 in response to ceramide in MB leprosy patients resulting in the suppression of Th1 response to *M. leprae.* However, the mechanisms by which the cellular immune response is so extraordinarily titrated as to produce the entire leprosy spectrum is still not clear. Other

Table2: Anti ceramide antibody titre in controls, paucibacillary and mutibacillary leprosy cases

Parameter	**Controls Mean ± SD**	**PB Leprosy Mean ± SD**	**MB Leprosy Mean ± SD**
Anti-ceramide IgM antibody titre (OD units)	0.148 ± 0.084	0.302 ± 0.172*	0.559 ± 0.396*†

* significant in comparison to controls
† significant in comparison to PB leprosy cases

Figure 3. ROC curve for serum anti-ceramide antibody titre in paucibacillary leprosy patients versus controls



Area under the curve 0.742 At cutoff, 0.259 sensitivity is 96% and specificity is 52%

than glycolipids of the myelin sheath, antibodies to sulfatide have also been reported in various demyelinating peripheral polyneuropathies [7]. Few groups of researchers have reported the role of these antibodies in leprosy in terms of their diagnostic value [11].

Thus the authors of the present study suggest that a correlation exists between higher serum anti-ceramide antibody titre and MB leprosy. MB leprosy is associated with nerve damage. Elevated anti-ceramide antibodies in MB leprosy patients as compared to PB leprosy patients clearly indicate their role in nerve damage and open a new dimension to the development of novel diagnostic markers in leprosy research. The data of the present study agree with the literature; however, the literature contains a limited number of studies. The estimation of ceramide antibodies is an indirect method to assess the effect on the glycolipid. The authors of this study would also suggest that the role of direct estimation of ceramide as a marker of myelin alterations be explored.

Conclusion

Research in the field of immunological aspects of leprosy continues on the premise of improving the understanding of complex immunoregulatory mechanisms related to the disease. Complications resulting from delays in the diagnosis and treatment of leprosy may be curbed by the search for biomarkers that can help to assess nerve damage. Anti-ceramide antibodies can be one such biomarker.

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