Evaluation of Serum Anti-Ceramide Antibody Levels in Egyptian Leprosy Patients

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Abstract

Background: Leprosy is a chronic granulomatous disease affecting skin and nerves. The nerve damage that occurs in leprosy is the most serious aspect of this disease as nerve damage leads to progressive impairment and disability. It is important to identify markers of nerve damage so that preventive measures can be taken.

Objective: To evaluate anti-ceramide antibody as a reliable marker in the assessment of nerve damage in paucibacillary and multibacillary leprosy patients.

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

Results: MB leprosy patients had significantly higher ($p<0.001$) anti-ceramide antibody serum levels compared to PB leprosy patients and healthy controls (0.633±0.150, 0.345±0.099 and 0.143±0.058, respectively). The serum level of ACA was significantly correlated with the duration of the disease in both PB and MB groups ($r=0.676$ & $0.653$; $p<0.001$) respectively.

Conclusions: It is important to identify markers of nerve damage in leprosy patients so that preventive measures can be taken. Anticeramide antibodies can serve as a marker for nerve damage by showing the extent of nerve damage, allowing for better management.

Key Words: Anticeramide antibody – Nerve damage – Leprosy.

Introduction

LEPROSY is the most common treatable peripheral nerve disorder worldwide. Deformity and disability result from nerve-function impairment. Early detection and treatment of nerve-function impairment are crucial for outcome, so special emphasis is given to developments in early detection and treatment of nerve impairment in leprosy [1].

Ceramide is a glycosphingolipid and is a major component of nerves, playing an important role in maintaining the integrity of neuronal tissue and also acting as a second messenger in signal transduction pathway leading to apoptosis. Categorizing the circulating plasma antibodies in leprosy indicated the presence of antibodies to ceramide suggesting its role in nerve damage [2].

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 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. 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 [3]. Aiming to evaluate the possible role of anti-ceramide antibodies (ACA) as a reliable marker in the assessment of nerve damage in leprotic patients, this study was done to assess anti-ceramide antibodies levels and to correlate their levels with type of leprosy, duration, nerve damage and disabilities in paucibacillary and multibacillary leprosy patients.

Patients and Methods

This study included 50 patients with leprosy (25PB and 25MB) recruited from Benha University Hospital and Benha Leprosy & Dermatology Clinic from May 2012 – May 2013. The control group consisted of 50 age-and sex-matched healthy individuals. Patients on drug therapy, immunosuppressive therapy such as corticosteroids, regular analgesics
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and/or with a history of inflammatory, autoimmune disease or any other systemic illness were excluded from the study. All participants were fully aware of the purpose of the study and gave verbal consent. The study was approved by ethics committee of Benha faculty of medicine.

Clinical assessment:
All patients were subjected to thorough history taking and clinical examination of the skin and nerves for enlargement and/or tenderness. 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.

Blood sampling:
Venous blood samples (5ml each) were collected from both leprosy patients and healthy controls under sterile conditions. Blood was collected in serum separator vacutainers and left for 20min at room temperature to clot and then centrifuged for 10min at 5000rpm to separate the serum. Serum samples were stored at –20°C until subsequent analysis.

Measurement of anticeramide antibody:
Anti-ceramide antibody was estimated using is a solid phase sandwich ELISA technique. It utilizes a monoclonal antibody (capture antibody) specific for human ACA coated on a 96-well plate. Standards and samples are added to the wells, and any human ACA present binds to the immobilized antibody. The wells are washed and biotinylated polyclonal anti-human ACA antibody (detection antibody) is added.

After a second wash, avidin-horseradish peroxidase (avidin-HRP) is added, producing antibody-antigen-antibody sandwich. The wells are again washed and a substrate solution is added, which produces a blue color in direct proportion to the amount of human ACA present in the initial sample. The stop buffer is then added to terminate the reaction. The result is a color change from blue to yellow. The wells are then read at 450nm [4].

Statistical analysis:
The collected data were tabulated and analyzed using SPSS version 16 software. Categorical data were presented as number and percentages while quantitative data were expressed as mean & standard deviation or median & range. Chi square test ($X^2$), iZ$^*$ test Student 't' test, Mann Whitney U test, ANOVA and Person’s product correlation coefficient ($r$) were used as tests of significance. ROC curve was used to detect a cut off value of serum anti-ceramide antibodies titre with optimum sensitivity and specificity. The accepted level of significance in this work was stated at 0.05 ($p<0.05$ was considered significant).

Results
Fifty leprosy patients, twenty-five PB patients (16 men and 9 women; average age, 30.96±12.5yrs [range, 7-52yrs]) and twenty-five MB patients (15 men and 10 women; average age, 33.12±13.9yrs [range, 11-61yrs]) were included in this study. The control group consisted of 50 healthy individuals (33 men and 17 women; average age, 31.22±8.9yrs [range, 17-49yrs]). The patients and controls were matched according to age and sex, and no statistically significant difference was noted between patients groups and controls using ANOVA test ($p>0.05$). As the duration of the disease was found to be positively skewed, so median and interquartile range are more appropriate than mean and SD. Using Mann-Whitney U test the median & IQ range was 1y & 10m-2.5y and 9m & 1m-10y in PB and MP groups, respectively (Table 1).

Higher levels of serum anti-ceramide antibody level was observed in 24 (96%) out of 25 cases of MB leprosy and in 20 (80%) out of 25 cases of PB leprosy.

As shown in Table (2), serum anti-ceramide antibody level was significantly higher in MB (0.633±0.150) than its level in PB and controls (0.345±0.099, 0.143±0.058) respectively, also it was significantly higher in PB than its level in controls.

No correlation was found between serum anti-ceramide antibody (ACA) level and both age and gender in both groups (Table 3).
The serum level of ACA was significantly correlated with the duration of the disease in both PB and MB groups ($r=0.676$ & 0.653; $p<0.001$) respectively (Fig. 1).

There was a positive correlation between nerve enlargement and serum ACA level (Table 4).

Table (3): Correlation between serum anti-ceramide level (ACA) and both gender and age.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Serum anti-ceramide level</th>
<th>Mean ±SD</th>
<th>Mean ±SD</th>
<th>ANOVA $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.347</td>
<td>0.107</td>
<td>0.653</td>
<td>0.178</td>
</tr>
<tr>
<td>Female</td>
<td>0.341</td>
<td>0.091</td>
<td>0.602</td>
<td>0.094</td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

The ROC curve could detects nerve damage in MB cases, at cut off value (0.610 OD) of serum ACA, with sensitivity 80%, specificity 80%, accuracy 85.3% and area under curve was 0.85. While ROC curve could detects nerve damage in PB cases, at cut off value (0.323 OD) of serum ACA, with sensitivity 91.7%, specificity 85%, accuracy 96.8% and area under curve was 0.97 (Table 5, Fig. 2).

Fig. (1): Correlation between serum ACA and duration of the disease in both groups.

<table>
<thead>
<tr>
<th>Serum Anti-ceramide antibody</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>AUC</th>
<th>95% CI of AUC</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB Cut off value =0.610</td>
<td>85.3%</td>
<td>0.85</td>
<td>0.71-1.0</td>
<td>0.003</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PB Cut off value =0.323</td>
<td>96.8%</td>
<td>0.97</td>
<td>0.91-1.0</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. (2): ROC curve showing the performance of serum ACA as a marker for nerve damage among MB & PB groups.
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Discussion

Humoral responses against nerve antigens are pathogenic in some peripheral and sensory peripheral neuropathies, and may also have a role in leprosy nerve damage [5]. Ceramide and other sphingolipids are recognized as lipid mediators of the immune response and high concentrations of auto-antibodies to neural proteins and lipid antigens have been demonstrated in leprosy patients [6].

In the present study higher levels of serum anti-ceramide antibody was observed in 24 (96%) out of 25 cases of MB leprosy and in 20 (80%) out of 25 cases of PB leprosy. Anti-ceramide antibody level was significantly higher in MB leprosy patients in comparison to both controls and PB leprosy patients.

Our result was in agreement with Singh et al., [3], Vemuri et al., [7], Park et al., [8] and Vemuri et al., [9]. Singh et al., [3] reported that a correlation exists between higher serum anti-ceramide antibody titre and MB leprosy. MB leprosy is associated with nerve damage and indicates that M. leprae infection is a major factor in inducing anti-neural antibody response.

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.

Narayan et al., [10] in their study have documented that 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, but they found no significant difference in the prevalence of antineural antibodies between PB and MB leprosy patients.

Anticeramide antibodies can serve as a marker for nerve damage by showing the extent of nerve damage, allowing for better management.

In our study there was a statistical positive correlation between serum anti-ceramide antibody level and duration of disease.

We noticed that patients had normal ACA levels (0.232, 0.227OD) had the shortest (1-2 month) duration of the disease in both MB and PB groups. While the greatest level of ACA (0.916 OD) were found in MB patient with longest (10 years) duration of the disease. This was in agreement with a study of Jadhav et al., [6], which was done on 303 multibacillary leprosy patients for 2 years and reported that ceramide antibody was elevated in patients with old sensory nerve damage. One explanation for this might be that auto-antibodies are not implicated in the initiation of new nerve damage, but once the nerve is damaged, then various nerve antigens may be presented to the immune system, initiating an auto-immune response. This may be one mechanism whereby nerve damage is perpetuated.

The present study showed that there was a statistical positive correlation between nerve enlargement and serum anti-ceramide antibody level. Our result was in agreement with the studies of Raju et al., [2]; Vemuri et al., [7] and Thomas et al., [11].

Raju et al., [2], reported that treated leprosy patients continue to have significantly elevated ceramide antibody level as compared to healthy subjects. This evidence of increased autoantibodies to glycosphingolipid components of nerve in treated
leprosy patients indicates that nerve damage continues as an autoimmune response to multiple nerve components even after a patient has completed leprosy treatment [2].

Our study showed that the ROC curve could diagnose nerve damage in MB cases, at cut off value (0.610 OD) of serum anti-ceramide antibody, with sensitivity 80%, specificity 80%, accuracy 85.3% and area under curve was 0.85, while the ROC curve could diagnose nerve damage in PB cases, at cut off value (0.323 OD) of serum anti-ceramide antibody, with sensitivity 91.7%, specificity 85%, accuracy 96.8% and area under curve was 0.97.

These results were close to results of Singh et al., [3], who reported that 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, 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 <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 [3].

Evaluation 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 controlled by the search for biomarkers that can help to assess nerve damage. Anti-ceramide antibodies can be one such biomarker.

References