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Immune Response to Mycobacterial Antigen in Cases of Guillain Barre' Syndrome

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Shripad A Patil .

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- *Department of Microbiology, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Arun B Taly, - *Department of Neurology, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

P Sowbhagya, - *Department of Neuropathology, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Ashok Menon &, - *Department of Neurology, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

A Chandramuki, - *Department of Microbiology, National Institute of Mental Health & Neuro Sciences, Bangalore 560 029, India*

Abstract

Guillain - Barre syndrome (GBS) is an immune mediated acute polyradiculopathy of undetermined etiology. A range of probable etiological agents such as viruses, bacteria, malignancy, surgery etc. have been suspected for the condition. In view with the endemicity of mycobacterial infections an attempt has been made to look for antimycobacterial immune response in the serum of patients with GBS. Anti-Mycobacterial antibodies of both IgG and IgM type are significantly high in serum of GBS than in controls ($p < 0.05$). Immune complexes which generally reveal the acute phase of infection were absent both in GBS and controls. The study indicates the probability of chronic association of mycobacterial infection in GBS cases.

Key words -

Guillain Barre' Syndrome,
Mycobacteria,
Antibodies,
Immune complexes

Mycobacteria are almost ubiquitous in nature. Mainly there are two pathogenic species of Mycobacteria (M. tuberculosis and M. leprae) to man and these cause tuberculosis and leprosy respectively. Tuberculosis is one of the commonest diseases of developing countries like India. However, with the advent of AIDS, an increase in number of cases of tuberculosis is reported from deveoped countries too [1]. India being endemic to tuberculosis, every individual is likely to be exposed to tubercle bacilli at some time in life.

Guillain-Barre' syndrome (GBS) is the commonest cause of acute polyradiculoneuropathy. It is believed that in many

patients GBS is preceded by a variety of viral, bacterial and mycoplasmal infections [2], [3], [4]. Keeping in view the endemicity of tuberculosis, in the present study an attempt has been made to estimate antimycobacterial antibodies in the sera of patients with GBS and establish possible association between Mycobacterial infection with GB syndrome.

Material and Methods

Five ml of blood was collected during the acute phase of illness (with 4 weeks of onset) from 36 patients fulfilling the current diagnostic criteria for GBS [5]. Chest X-rays were examined to exclude the possibility of pulmonary tuberculosis. Blood was also collected from 41 healthy blood donating controls. Serum was separated and stored at-20° C till used.

Anti-mycobacterial Antibody Test (ELISA)

ELISA was done per the method of Chandramuki et al [6] with little modifications. Microtiter ELISA plates (Immulon flat bottom, Dynatech, U.S.A) were coated overnight with 50µ l/well (10µ g/ml) of M. tuberculosis soluble extract as antigen and washed with phosphate buffered saline (PBS). Free sites of the wells were blocked using 1% milk in PBS with 0.05% Tween 20 (Sigma, U.S.A). Plates washed and later incubated with 50µ l of 1:500 diluted serum in duplicate for two hours at 37° C. Plates washed again (× 5) and further incubated with 50µ l well of peroxidase conjugated anti-human IgG (1:4000, Dakopatts, Denmark), IgM (1:1000 Dakopatts) for two more hours. Plates were later washed (x5) and developed with o-phenylene di-amine (Sigma, U.S.A.) chromogen in 0.1 M citrate phosphate buffer (pH-5) containing 0.01% hydrogen peroxide as substrate for 10min. The reaction was stopped with 50µ ml/well of 4N sulphuric acid and optical density (O.D.) of the colour was read at A492. Samples showing OD of more than 0.400(mean + 2SD of controls = 0.394) were taken as positive for IgG type of antinbodies, For IgM type, keeping in view the pentamer nature of the antibody molecule and due to sudden augmentation of the antibody signal even at minimal immune response a safer cut off point (mean + 4SD = 0.371) has been taken at OD, 0.400.

Mycobacterial immune complexes

Immune complexes were detected as per the method of Patil et al [7] with little modification. In this assay, flexible PVS microtiter plates (Dynatech, U.S.A.) were coated with 50µ l per well of anti-BCG antibody (Dakopatts Denmark, 1:1000 of 3.15 gm%) by over night incubation at 4° C. Free sites were blocked using 2% milk (Anik spray, India) in phosphate buffered saline (PBS, pH 7.4) at 37° C for two hours. Later, plates were incubated with 50µ l/well of 1:500 diluted serum samples in 1% milk for two hours. The plates were washed (× 5) and further incubated with 50µ l/well of peroxidase conjugated anti-human IgG (1:4000, Dakopatts)/ IgM (1:1000 Dakopatts) for two more hours at 37° C. Later plates were washed and developed with o-phenylene di-amine (Sigma, U.S.A.) in 0.1 M citrate Phosphate buffer (pH-5) containing 0.01% hydrogen peroxide for 10 min. The reaction was stopped with 4N sulphuric acid and optical density (O.D.) was read at A 492. Samples showing OD of more than 0.400 (mean + 2 SD of controls = 0.374 for IgG; mean + 4 SD = 0.405 for IgM) were taken as positive for the assay.

Results

Anti-microbacterial antibodies of IgG type were detected in 9 of 36 (25%) patients of GBS, where as antibodies of IgM type were noticed in 4 of 36 (11%) patients during acute phase of GBS (Fig .1). Mycobacterial immune complexes of both IgG and IgM type were absent in the serum of all the patients.

Scattern of mycobacterial immune response in GBS and controls

Serum samples of healthy blood donors, which served as controls were positive for anti-mycobacterial antibodies of IgG type, in 4 of 41 (10%) subjects, whereas, none was positive for IgM type of antibodies. Mycobacterial immune complexes of both IgG and IgM type were absent in control group as well.

Discussion

Triggering of autoimmune process is quite common in Mycobacterial infections, thus leading to the synthesis of auto-anti-bodies to various cellular components [8]. Heat shock proteins which are ubiquitously present [9] are produced abundantly in Mycobacteria during stress and are generally accountable for auto-immune disorders because of the molecular mimicry between heat shock proteins of micro-organisms and cellular self components [10], [11].

Guillain-Barre syndrome is one of the commonest cause of acute poly radiculoneuropathy of undetermined etiology. The underlying pathogenetic mechanism is immunologically mediated damage to peripheral nerve myelin. A wide range of etiological agents such as viral and bacterial infections, surgery, malignancy, vaccination and drugs have been associated with GBS [4]. Immune complexes have also been implicated in the pathogenesis of GBS [12].

The Chi-square test of goodness of fit revealed that the presence of anti-Mycobacterial antibodies in the serum of GBS patients is significantly higher ($p < 0.05$) than in control sera for both IgG and IgM type of antibodies.

Mycobacterial immune complexes which are generally detected earlier than free antibodies are indicative of recent infection [7]. Absence of mycobacterial immune complexes and positivity for antimycobacterial antibodies in sera of GBS patients probably indicates chronic association of mycobacterial infection in GBS patients. Because of the chronic and latent type of mycobacterial infection, difficulties were encountered in the selection of two sampling periods to see rise or fall in anti-mycobacterial antibody titer. It needs to be further explored whether mycobacterial infection predisposes the patients to GBS because of immune alteration.

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