Ig A Antibodies to Klebsiella in Ankylosing Spondylitis

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Summary

In this study anti-klebsiella Ig A values were compared in 40 patients with definite diagnosis of ankylosing spondylitis and a control group of 40 healthy subjects. Anti-Klebsiella Ig A antibody values were significantly higher in patients with ankylosing spondylitis as compared to the control group (p < 0.001). Correlation between these antibodies and erythrocyte sedimentation rate, CRP, serum Ig A, HLA B 27, age, sex and disease duration was searched, but no correlation was found. In our opinion, these results support the suggestion that inflammatory response in ankylosing spondylitis is triggered by Klebsiella but is insufficient to prove the causal relationship between ankylosing spondylitis and Klebsiella.

Key words

Ankylosing Spondylitis, Klebsiella

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, progressive, inflammatory disease that primarily affects vertebral and sacroiliac joints. This disease causes development of deformities and progressive limitation of mobility, and has an aetiology still under current discussion. Genetic and environmental factors as well as several microorganisms have been considered as responsible for the initiation of the disease. The opinion extensively agreed upon is that among these microorganisms klebsiella (K) is the cause of ankylosing spondylitis via a cross-reaction due to similarity of molecular structure with HLA B27 (1).

Klebsiella is an immobile, capsuled, gram (-) bacterium that is found both in the upper respiratory tract and intestines in humans and animals (2). Isolation of K in faeces of AS patients has been reported for many years (3-5). Furthermore, many studies have demonstrated the presence of antibodies against klebsiella in AS patients. In these studies, the methods used were ELISA (6,7), immunoblotting (8), bacterial absorption (9), and indirect Coombs' test for bacterial agglutination (10).

The aim of this study was to evaluate the relationship between K and AS in the Turkish population which has a different genetic background compared to the previous literature. In this study, the presence of Ig A antibodies against Klebsiella was investigated in ankylosing spondylitis cases as well as in healthy individuals while the relationship between presence of IgA antibodies and several activation parameters such as erythrocyte sedimentation rate, CRP, or serum IgA levels were examined. Hence, the role that klebsiella plays in the disease was discussed.

MATERIALS AND METHODS

This study was carried out by the physical Medicine and Rehabilitation Department of A.Ü.T.F. ibn-i Sina Hospital. Ten (25 %) female and 30 (75 %) male of a total 40 AS patients were included in the study, and 1966 New York diagnostical criteria (11) were applied to patients for definite diagnosis of disease. Age range and the mean age of patients were 20-65 and 36.82, respectively. Mean duration of illness was 11.22 years. Forty healthy individuals, who were determined as having no disease according to clinical and laboratory examination results were also included in the study as the control group. The control group consisted of 12 (30 %) females and 28 (70 %) males with a mean age of 35.25 (19-65).

In the study, the erythrocyte sedimentation rate (ESR) (applying Westergren method, mm/h), C-reactive protein (CRP) (mg/L), and IgA levels (g/L) of all patients were measured, and also the presence of HLA B27 was investigated. In addition to these, analyses of leukocytes, erythrocytes, haemoglobin, haematocrit, routine blood biochemistry, and urine were performed in the patient group. In the control group, ESR, CRP, IgA levels were
assessed and routine blood and urine analyses were performed as well.

Serum anti-klebsiella IgA (anti-K IgA) antibodies were examined with enzyme-linked immunosorbent assay (ELISA) method for both patient and control groups. ELISA was applied in the manner defined by Voller et al. (12) using the modifications given below.

**Preparation of antigens and the coating of plates**

The amount of carbohydrate in cell lysate derived from klebsiella pneumoniae (Kp) K43 strain was assessed by the Dubois method (13). ELISA plates consisting of ninety-six wells were coated in a carbonate-bicarbonate (pH=9.6) buffer solution so as to have 10 ug glucose per well and were suspended overnight at +4° C. They were washed 5 times with a phosphate buffer solution containing twin 20 (PBST). Then they were coated with PBST of 1% bovine serum albumin in order to block possible nonspecific attachments. Again they were washed 5 times with PBST and were incubated for 2 hours at 37°C with patient serum diluted to a 1:10 ratio. Following that, they were washed 5 times. As the second antibody, horse radish peroxidase anti-human IgA was used and the incubation was continued an hour longer at 37° C. Following wash-up 5 times with PBST, a substrate mixture containing orthophenilene diamine was added and it was allowed to remain 20 minutes in the dark for the reaction to take place. The reaction that occurred as a result was detected by ELISA detector at 490 nanometers. All samples were double-studied. The statistical analyses of the results were performed by applying the Mann Whitney U test for ELISA of anti-klebsiella IgA antibodies and the chi-squared, and Fisher-Exact test.

**RESULTS**

These optical densities of each group are presented in a dot diagram (Table I).

According to statistical analysis, the values of anti-klebsiella IgA antibodies in AS patients as compared to the control group were found to be statistically significant (p < 0.001).

Table I: Anti-klebsiella Ig A values in the groups.