Immune responses to outer membrane proteins and lipopolysaccharide of Salmonella Typhimurium and their relations to enteric reactive arthritis.

Abdal Razak H. Ahmed*, Mohammed Hadi** and Jabbar Salman Hussan**

The abstract

Arthritis is a chronic, disabling, and acute disease that develops during infection or following infection, in a part of the body; in particular, the reactive arthritis or bowel and urinary tract. It is not possible to isolate the infectious agent from the affected joint, arthritis and in many cases, is more severe.

The study aims to achieve the best antigen and the best antibody that help to diagnose the disease and relationship between the immune response and the severity of the disease.

The study included five patients suffering from reactive arthritis, who were admitted to the Medical City hospital in Baghdad, in addition to three referee cases of reactive arthritis. By taking a blood sample from each patient, serum was separated, while the blood was observed. A test was conducted to determine the antibodies of the outer membrane, and the antibody of the Salmonella typhimurium was multiply antigenic.

From these patients, twenty and twenty women, and twenty and twenty men with repeated, we found that the antibody of the outer membrane was present in 80% of the cases. The study showed that the reactions found in the study were significant, and the antibody of the outer membrane of Salmonella typhimurium was not significant. However, the study showed that the antibody of the outer membrane of Salmonella typhimurium was positive in cases of enteric reactive arthritis.

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**Abstract**

**Objectives:** In the present work, we analyzed the predominant Salmonella typhimurium component outer membrane proteins and lipopolysaccharide (OMPs and LPS) that triggered an immune response in 45 patients with enteric reactive arthritis by assessing the anti-OMPs and anti-LPS antibodies (including IgG, IgM, and IgA) by ELISA.

**Methods:** Forty-five patients with reactive arthritis, they were 22 (48.89%) males and 23 (51.11%) females, the age range was 20 - 40 year with mean (33.6). All patients were outpatient visitor or hospitalized in city hospital in Baghdad over the period of study. They were diagnosed clinically by consultant rheumatologist, as well as some laboratory tests such as RF, CRP, and ESR.

The patients should be sero-negative (RF-negative) and fulfill Amor and European Criteria (1), to be included in this study, patients were classified according to disease activity in to three group severe, moderate, and mild by using (DAREA score)(2), the majority of patients 19 (42.22%) presented with high disease activity (severe) and 15 (33.33%) patients were moderate and the remainder were mild disease group consist of (11) patients (24.44%). Thirty age and sex matched apparently healthy individual, were considered in this study as a control group. Wells were coated with antigens (OMPs and LPS of Salmonella) in coating buffer and anti-OMPs and anti-LPS antibodies were assayed using ELISA technique.

**Results:** The mean age of patients was (33.6) years and there were 23 females and 22 males with females to male ratio 1.05:1, the majority of patients 19 (42.22%) present with high disease activity (severe) and 15 (33.33%) patients were moderate and the remainder were mild disease group.

Three classes of antibodies to OMPs and LPS antigens were studied by ELISA. Positive responses to OMPs and LPS in serum of patients were detected and the major antigenic target in Salmonella-induced ReA was LPS and the main antibodies were IgG anti-LPS. Also there was no significant difference between severe, moderate, and mild among ReA patients.

**Conclusion:** We concluded that LPS were the main bacterial antigens that triggered enteric ReA in this study, and determining the triggering bacterial components can help elucidate the precise causes of ReA and will contribute to the designing of a specific serological diagnostic method for this arthritis.
**Key Words:** Reactive arthritis, heat shock proteins, Development of a disease activity index for the assessment of reactive arthritis (DAREA)

**Introductions**

Reactive arthritis (ReA) is a synovitis developing after a distant infection usually in the genitourinary or gastrointestinal tract which suggest a contribution from bacterial product (3), but the organism can not be isolated or cultured from the joint (4). Many Gram negative bacteria including *Chlamydia trachomatis*, *Shigella*, *Salmonella*, *Yersinia* and *Campylobacter* have been implicated in the underlying pathogenesis of ReA (6) ReA affects male and females with same frequency (5). However, it was previously claimed to be more common in males, and most patients are aged between 20-40 years (6) and the exact etiology of ReA is unknown. However genetic factors play a role in susceptibility to the disease and 65-80% of patients are positive for HLA –B27, and many infections may be implicated in the etiopathogenesis of ReA (5). At the time of arthritis, stool cultures are usually negative, and the background of ReA has usually been confirmed by serological method (7).

There are two hypothesis explain develops of ReA in HLA-B27 positive subjects (8).

The first is the arthritogenic peptide hypothesis: which suggest that the arthritis is triggered by a T-cell response to specific antigenic peptides derived from the triggering bacteria. The other hypothesis is molecular mimicry hypothesis: this theory postulates that an autoimmune process can ensue after an infection if there is some degree of cross-reactivity in host and microbial antigens (9).

*Salmonella typhimurum* is a major cause of food –borne illness (4).is the most frequently detected causes of outbreak of human Salmonellosis(10), and a high frequency of ReA has been observed after infection with this bacteria (11)

**Patients and Methods**

This study included forty-five patients with Reactive arthritis, they were 22(48.89%) males and 23(51.11%) females, the age rang was 20 - 40 year with mean (33.6). All patients were outpatient visitor or hospitalized in medical city hospital in Baghdad over the period of study They were diagnosed clinically by a consultant rheumatologist, as well as some laboratory tests such as RF, CRP and E.S.R.

The patients should be sero-negative (RF-negative) and fulfill Amor and European Criteria (1) to be included in this study. The
patients with definite history of diarrhea were grouped as enteric reactive arthritis. (3.5 ml) blood were collected and transferred into a plan tube allowed to clot at room temp. Then centrifuged 15 minutes approx. 500 rpm to obtain unhemolyzed cell –free serum. Serum sample were divided in aliquots at -20°C to avoided freezing and thawing in each steps of this study. Thirty (age and sex matched) apparently healthy individual, were considered in this study as a control group, all control persons had no history of diarrhea.

Lipopolysaccharide (LPS) extraction was obtained by LPS extraction kit which is designed for rapid and convenient extraction of LPS from bacterial cell the extraction procedure is take only 60 minute and give high yields of LPS, While (OMPs) extraction process making according to (Murphy, et al., 1983 and Hunter, et al., 1986)(12,10)

The protein concentrations of the (OMPs) were determined Biuret method, the concentration of protein in OMPs of Salmonella was 0.9 mg/ml by using (BSA) standard curve, and the estimation of LPS concentration (Ketodeoxyoctinate) by using Thiobarbituric acid assay(13) the concentration of LPS in Salmonella it was 112 microgram /ml by using LPS standard curve.

Procedure of ELISA was making according to the Hunter, et al 1986 (10) as following:
Wells were coated over night at 4°C with 100µ of 1/40 diluted antigens in coating buffer ,Next day the plates were emptied and washed three times with washing buffer .then uncoated sites were blocked with 100µ/ well blocking buffer for one hour at 37°C incubation was carried out in a shaker incubator. Then plates were emptied and 100µ of 1/2 diluted sera in dilution buffer were added to each well and the plates incubated for 1hr. at 37°C. Then the excess non –reacted sera removed through three cycle of washing with washing buffer while the reacted sera detected by adding to each well 100µ of 1/1000 diluted conjugate and plates incubated for 1hr.at 37°C. (The conjugated used were):

-Horse raddish peroxidase-anti human IgG conjugate
- Horse raddish peroxidase-anti human IgM conjugate
- Horse raddish peroxidase-anti human IgA conjugate

After incubation the plates were washed with washing buffer and 100µ of substrate solution (OPD) were added to each well and incubated in dark place for 30 minutes at 37°C and the reaction was stopped by addition of 100µ stopping solution and the absorbance was determined with an ELISA reader at 550 nm.
An optical density (OD) value of more than mean plus two standard deviations of normal control was considered as positive.

Results

The study included 45 patients with enteric ReA. And 30 age and sex matched apparently healthy control. The mean age of the patient was (33.6) years with range from 20-40 years the highest incidence of ReA was in 4th decade followed 3rd decade. Figure (1)

There were 23 females and 22 males with females to male ratio 1.05:1. Chi –square revealed no statistically significant difference in the frequency of patients between both sex (P=0.763) this mean there was no significant sex effect. Although; there was a slight inclination for an association with female sex.

When all class antibodies to OMPs and LPS of Salmonella were studied by ELISA, we detected positive response to OMPs and LPS in serum of most patients, the highest antibody response were the response against LPS, in addition a higher Salmonella- specific IgA response was detected against OMPs compare with anti-OMPs IgM and anti-OMPs IgG, In contrast we found that the IgG antibody response was stronger to LPS compare with anti-LPS IgM and anti-LPS IgA.
Table 1: Comparison between reactive arthritis patients group regarding immune response against OMPs and LPS.

<table>
<thead>
<tr>
<th>Immunoglobulin type</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM anti- OMP</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>IgA anti- OMP</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>IgG anti- OMP</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>IgM anti- LPS</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>IgA anti- LPS</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>IgG anti- LPS</td>
<td>3</td>
<td>42</td>
</tr>
</tbody>
</table>

In this study there is no significant difference between immune response to OMPs and LPS regarding disease activity group.

Table 2: Comparison among ReA patients according to humoral immune response regarding the disease activity.

<table>
<thead>
<tr>
<th>Salmonella</th>
<th>IgM anti-OMP</th>
<th>IgG anti-OMP</th>
<th>IgA anti-OMP</th>
<th>IgM anti-LPS</th>
<th>IgA anti-LPS</th>
<th>IgG anti-LPS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mild</td>
<td>moderate</td>
<td>severe</td>
<td>total</td>
<td>mild</td>
<td>moderate</td>
</tr>
<tr>
<td>Salmonella IgM anti-OMP Negative</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>22</td>
<td>0.384</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgM anti-OMP Positive</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>23</td>
<td>0.384</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgG anti-OMP Negative</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>12</td>
<td>0.268</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgG anti-OMP Positive</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>33</td>
<td>0.268</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgA anti-OMP Negative</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0.649</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgA anti-OMP Positive</td>
<td>9</td>
<td>14</td>
<td>17</td>
<td>40</td>
<td>0.649</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgM anti-LPS Negative</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>Salmonella IgM anti-LPS Positive</td>
<td>11</td>
<td>12</td>
<td>14</td>
<td>37</td>
<td>0.185</td>
<td></td>
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<tr>
<td>Salmonella IgA anti-LPS Negative</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>21</td>
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<td>Salmonella IgA anti-LPS Positive</td>
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<td>7</td>
<td>11</td>
<td>24</td>
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<tr>
<td>Salmonella IgG anti-LPS Negative</td>
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<td>1</td>
<td>3</td>
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<tr>
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<td>13</td>
<td>18</td>
<td>42</td>
<td>0.834</td>
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</table>
**Discussion**

Three classes of antibodies (IgG, IgM and IgA) to LPS and OMPs of Salmonella typhimurium were studies by ELISA, We detected positive response to OMPs and LPS for Salmonella compared with healthy control group. In addition, the highest antibodies were response against LPS of Salmonella. Indicating that they were the major antigenic target in Salmonella induced ReA disease in our studies compare with OMPs.

Also we found higher Salmonella specific Immunoglobulins was IgA against OMPs antigen (IgA anti-OMPs of Salmonella). That suggests continuous antigen stimulation following Salmonella infection this quite accord with abroad studies, (Sukumar, et al., 2000; Hannu, et al., 2004).

In addition the highest Salmonella specific Immunoglobulins was IgG against LPS antigen (IgG anti-LPS of Salmonella). This disagreed with other previous studies done by Maria and his Co-worker (2007) who found that OMPs is the major antigenic target in Salmonella induced ReA, while the present results come in agreement with those data reported by (Maki, et al., 1991) (14)

This study observed that there was no correlation between the immune response to the (LPS and OMPs) and disease activity. And that may be due to the disease activity rate influenced by many factors like genetic of host, resistant to antibiotic; in addition the patients came in late state.

**References**