Detection of Anti-Lysozomal Antibodies and Beta-2-Microglobulin Protein in Patients with Typhoid Fever

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Abstract: Infection with many microbial agents can cause aberration in immune response through molecular mimicry or prolonged stimulation of immune system. *Salmonella*, the causative agent of typhoid fever, may have this feature. This study aimed to explore the possible association of serum levels of anti-lysozomal IgG antibodies (ALIA) and beta-2 microglobulin (B2M) with typhoid fever. A total of 52 typhoid fever patients and 35 healthy controls were recruited for this study. From each participant, a blood sample was obtained from which serum was separated. Enzyme linked immunosorbent assay was used to estimate serum levels of anti-lysozomal IgG antibodies and B2M. Patients with typhoid fever had higher significantly serum levels of ALIA than control (5.136±3.345 U/ml and 2.946±3.257 respectively), while there was no significant difference in serum levels of B2M between the two groups (0.889±0.766 µg/ml and 1.173±0.81 µg/ml respectively). These results indicate that infection with *Salmonella* can cause increase in serum level of anti-lysozomal IgG antibodies but not beta-2 microglobulin.

Key words: typhoid fever, lysozyme, microglobulin, *Salmonella*, ELISA.

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Introduction

Typhoid fever is a common world-wide bacterial disease transmitted by the ingestion of food or water contaminated with the feces of an infected person or animal, which contain the causative agents; *Salmonella*. Three methods for naming salmonellae are currently used, the modest one of which is that based on the relatedness determined by DNA hybridization analysis (1). According to this method, all salmonellae properly belong to a single species (*Salmonella enterica*), while former species such as *S. typhi* and *S. paratyphi* are no longer still as distinct species but are classified as *S. enterica* serotype *typhi* and *paratyphi* respectively (2). Clinically, *Salmonella* species are categorized in two distinct categories; the typhoidal species (*S. enterica* serotype *typhi* and *S. enterica* serotype *paratyphi*) and nontyphoidal species which include many other serotypes of *S. enterica* especially *S. enterica* serotype *choleraesuis* (1).

Lysozyme is a glucosidase localized in the specific granules of neutrophils. It has antibacterial activity via hydrolyzing the β-(1,4) glycosidic bond between N-acetylmuranic acid and N-acetylglucosamine in peptide-glycan. This hydrolysis renders bacteria sensitive to lysis by turgor pressure. Most gram negative bacteria are not susceptible to the action of the lysozymes alone. That is because the outer membrane of these bacteria prevents the access of enzyme to the
peptidoglycan layer. However, the immune system can overcome this barrier via the production of antibacterial proteins that permeabilize the outer membrane, such as lactoferrin (3). Beside their specific pathogenic effects, infections can lead to formation of multiple antibodies (Abs), such as rheumatoid factors, antinuclear Abs (ANAs) and antineutrophil cytoplasmic Abs (ANCAs) including anti-lysozymal Abs (4). In their study, Locht et al. (5) found that ANCA was prevalent among patients with reactive arthritis (ReA), occurring in 56% in cases. The reported incidence of Salmonella-induced ReA is between 1.2% to 7.5% (6). The longer duration of the infection allows for more opportunities for the microorganism to enter the bloodstream and stimulates the immune system for production of such Abs (7). Antigenic cross reaction (molecular mimicry) and/or the prolonged exposure to neutrophil antigens has been postulated as the driving force for autoantibody production (5).

Beta-2-microglobulin is a small, 11.5 kDa polypeptide. It is a component of class I major histocompatibility complex and is, therefore, present on the surface of all nucleated cells (8). Clinically, serum levels of B2M can be used as a marker for cellular immune system activity as well as tumor marker in certain hematological malignancies such as multiple myeloma and lymphoma (9). Beyond these malignancies, Svatonova et al. (10) found high B2M levels in cerebrospinal fluid but not in serum of patients with bacterial meningitis, and the authors referred this elevation to inflammatory response.

Serum levels of ALIA and B2M protein in typhoid fever have not been studied before. Therefore, this study aimed to investigate the significant of these two factors in patients with typhoid fever.

Materials and Methods

This case-control study involved 52 outpatients (34 male and 18 female, mean age 41.13 years) diagnosed primarily as having typhoid fever in Al-Imamain Al-Kahumain Medical City / Baghdad / Iraq for the period from June 2013 to June 2014. The diagnostic criteria were either positive blood culture for S. enterica serotype typhi or S. enterica serotype paratyphi or a four-fold rise in antibody titer against these serotypes. Other 35 (24 male and 11 female, mean age 37.17 years) apparently healthy individuals were recruited to represent control group. Patients or control who had diagnosed with any hematological malignancy or autoimmune disease were excluded from the study. Five-milliliters (ml) of blood were taken from each participant in plane tubes which allowed to coagulate. Sera were separated by centrifugation and dispensed into 1 ml aliquots and stored at -20°C until be used.

Immunological Assays

Enzyme-linked immunosorbent assay (Diagnostic Automation Inc, USA) was used to estimate serum levels of anti-lysozyme antibodies and β-2-microglobulin using ready kits (Immuchem, Belgium) according to manufacturer's manual. Briefly, serum samples were diluted 1:10 with sample buffer, and 100 µl of each of calibrator, prediluted patients and control sera were pipetted into defined wells of the plate. The plate was then incubated at 20°C for 30 min and the contents were discarded. Wash solution was used to wash the plate 3 times, and 100 µl of enzyme conjugated solution was dispensed into each well. After 15
min incubation at 20° C, the contents were discarded from the plate which was again washed 3 times. One hundred µl of TMB substrate solution was dispensed into each well and 100 µl of stop solution was added to each well. The optical density was read at 450 nm. Diagnostic Automation apparatus is equipped with software that can read the concentrations directly without the need for absorbance values and standard curve.

**Statistical Analysis**

Statistical package for social sciences version 16.0 (Chicago, USA) was used to analyze the data. Significant differences in means of anti-lysozymal antibodies and B2M between typhoid fever patients and control group were performed using t-test of independence. The concentrations of the variables in the serum were expressed as means± standard deviation (SD). Statistical significance was set at a P value< 0.05.

**Results**

The most prevalent symptoms among typhoid fever patients were step-ladder pattern fever (78.84%), anorexia (61.5%), vomiting (28%), diarrhea (25%), headache (21.15%), and myalgia (19.2%). Other symptoms such as abdominal pain and constipation are also recorded but with minor occurrence.

**Anti-Lysozymal IgG Antibodies**

Serum levels of anti-lysozymal IgG antibodies in typhoid fever patients ranged from 0-10 U/ml with an average 5.136±3.345 U/ml. On the other hand, these values ranged from 0- 9.141 U/ml among healthy control with an average 2.946±2.312 U/ml with significant difference (t= 3.025, P= 0.003) (Figure1).

![Figure 1: Anti-lysozymal IgG antibodies concentration (U/ml) in serum of typhoid fever patients and control](image)
**Beta-2 Microglobulin**

Serum levels of B$_2$M from both typhoid fever patients and healthy control were within normal limits (< 3µg/ml) and the two means (0.889± 0.766 µg/ml and 1.173±0.81 µg/ml respectively) did not differ significantly (t=1.733, $P=0.087$) although the control group had high mean level (Figure 2).

![Beta-2 microglobulin concentration (µg/ml) in serum of typhoid fever patients and control](image)

**Figure 2: Beta-2 microglobulin concentration (µg/ml) in serum of typhoid fever patients and control**

**Discussion**

The current study was conducted to explore the possible association of typhoid fever and serum levels of ALIA and B$_2$M. The study revealed an increase in serum levels of ALIA in patients with typhoid fever. Unfortunately, there are no available similar previous studies for comparison, and the induction of ANCA by *Salmonella* received little attention. Autoimmune reaction which occurs post bacterial infections is a well known phenomenon. Among most studied examples are reactive arthritis proceeding the bowel infection with some pathogenic bacteria such as *Compylobacter* spp. And *Salmonella enterica*, and rheumatoid fever following infections with Group A streptococci (11). Probably, the infectious agents can trigger the formation of autoantibodies via many mechanisms. One of these mechanisms is the formation of inflammatory environment which facilitates the emergence of autoimmune processes and the generation of cross-reacting antibodies (12). The other mechanism is related to the autoimmune disorders following treatment with various antibiotics. However, when these drugs are removed from the body, the
corresponding auto antibodies disappeared (13). The first mechanism rather than the second probably associates with such increase in ALIA in typhoid fever patients. That is because the causative agent induces acute inflammatory response mediated by neutrophils. Neutrophils harbor an arsenal of antimicrobial defenses among which the oxidative burst and the generation of reactive oxygen species (ROS), and antimicrobial peptides involving lysozymes (14). Thus, it is reasonable to assume that persistent infection can induce larger amount of lysozyme. Some of these formed lysozymes may find its way to bloodstream, and, ultimately, autoantibodies will be formed against it. However, this hypothesis implies the formation of autoantibodies against other components of antimicrobial peptides, an assumption which needs to be confirmed in vivo. Serum levels of B2M are elevated in diseases associated with increased cell turnover and in several benign conditions such as chronic inflammation, liver disease, and renal dysfunction. These levels can elevate also in a number of malignancies, especially hematologic malignancies associated with the B-lymphocyte lineage (15). Free B2M can be found in different body fluids (include serum) under physiological conditions as a result of shedding from cell surfaces (16). High concentration of this protein was shown to affect the antigen presenting cells (APCs), especially dendritic cells. In such a condition, these cell suffer from many functional regressions among which the inhibition of up-regulation of surface expression of MHC class I, inhibition of nuclear factor-κB and diminishing in their ability to activate T cells (17). Therefore, increased serum concentration of this protein is supposed to inhibit the normal function of immune system. Accordingly, we hypothesized that typhoid fever patients might originally suffer from the presence of abnormal amount of B2M in their sera. However, the current study indicated no relation of typhoid fever with serum B2M.

References


