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Original Article

Evaluating the Level of Serum IL-23 in Brucellosis Infection by ELISA and Investigating its Relationship in Cases with Failure to Respond to Treatment

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Abstract

Brucella is belonging to the small immobile gram-negative spore-lacking cocco-bacilli bacteria family that grows in an aerobic environment, it is known as a zoonosis infection named brucellosis. This study was designed to investigate serum values of IL-23 in patient with brucellosis and investigate its relationship with cases with failure to respond to conventional medical therapy. A total of 372 individuals were divided into 2 groups (n=186) as follows: Group A comprising 186 infected participants with *brucella* (7-80 years-old), these people had not received antibiotics for at least 6 months ago. Group B including the healthy participants. All the participants in both groups were in the same age range. 5 ml blood samples were obtained from the participants intravenously (without anticoagulation substance). The serum level of IL-23 was investigated by ELISA diagnostic kit. The recorded data showed that the levels of IL-23 in the serum samples obtained from group A (143.64 Pg/ml) significantly (P<0.001) increased compared with this value in group B (23.14 Pg/ml). Based on the recorded data in the forms completed by all the participants at the day 0 of the experiment, 44 out of 186 individuals in group A, had experienced Brucellosis attack 2-3 times in spite of receiving medical prescriptions. A hypothesis about the possible immune system disorders in these participants lead us to did the re-sampling following drug administration. Results illustrated failure to respond to conventional medical therapy in patients with low level of serum IL-23.

Keywords: IL-23, Serum value, Brucellosis, Medical therapy

1. Introduction

Brucella is belonging to the small immobile gram-negative spore-lacking cocco-bacilli bacteria family that grows in an aerobic environment, it is known as a zoonosis infection named brucellosis (1). *Brucella* has several interesting characteristics as follows: it is

enduring against dryness, and it can survive in an environment with low temperature. Only strong disinfectants such as hypochlorite and formalin have the ability to kill this bacterium. Also, pasteurization can lead to the disinfection of this bacterium (2).

Brucellosis is a worldwide health issue that threatens human beings' lives, especially in the Mediterranean, Middle East, and Latin America areas (3-6). The incubation period of this bacterium varies. In humans, it causes febrile septicemia or localized infection (bone, soft tissues, or vital organ systems) (7). It is well documented that Brucellosis has variable incubation periods; it may take for 3 weeks to even several months. In severe brucellosis, the manifestation of the clinical symptoms are fever, chill, and muscular pains accompanied by severe perspiration, general restlessness, limpness, insomnia, headache, arthralgia, anorexia, and constipation.

Cytokines are known as hormone-like proteins that are secreted by a wide range of cells. It has been approved that cytokines are necessary for mediating immune responses (8). The presence of microbes and antigens in the living body triggers cytokine production. Each kind of cytokine exerts a distinct influence on the involved cells in immune and inflammation processes. Within induction of immune responses, cytokines stimulate the growth and differentiation of lymphocytes. On the other hand, during the operational stage of the inherent and acquired immunity, cytokines activate various executive cells enabling them to obliterate microorganisms (9). Also, cytokines can stimulate the creation of blood cells as well (10).

One member of cytokines group is called IL-23 which is a pre-inflammation cytokine belonging to IL-12 family (11). IL-23 plays a pivotal role in the induction of the production of some cytokines which are per se linked with Cell-mediated immunity (CMI). The most remarkable action of IL-23 and IL-12 cytokines is to induction of the production of IL-17A by Th17 cells. The IL-17A is belonging to the pre-inflammatory cytokines family (12). Similar to IL-12, IL-23 is released by dendritic cells or macrophages on which pathogens have had impacts (13). A package of cells and biological effectors such as CD4+ and CD8+T lymphocytes, T-helper 1 (Th1) type cytokines like (IFNγ) and TNFα, and activated macrophages and dendritic cells (DC) which together improve an intricate response against brucella have been known as the required factors for the host control of infection. It is well documented that the Th1 cytokines plays a key role in control brucella infection control. It has been approved that the IL-12 known as a critical bioactive molecule for the development of Th1 responses. It is mainly produced by antigen-presenting cells (APC). T cells are the main source of secretion for IL-10 which is an anti-inflammatory cytokine. This citokine interacts with IL-10 receptor and similarly to the signaling pathway of IFN-γ acts through the Jack/Stat signaling pathway. IL-10 is known to down regulate Th1 response during Brucellosis.

Therefore, this study was designed to investigate serum values of IL-23 in patient with brucellosis and investigate its relationship with cases with failure to respond to conventional medical therapy.

2. Materials and Methods

2.1. Participants

A total of 372 individuals were divided into 2 groups (n=186) as follows: Group A comprising 186 infected participants with *brucella* (7-80 years-old), these people had not received antibiotics for at least 6 months ago. Group B including the healthy participants. All the participants in both groups were in the same age range.

2.2. Blood Sampling

5 ml blood samples were obtained from the participants intravenously (without anticoagulation substance). The separated serum samples were kept at -70°C until the day of analysis. It is important to consider that the titers ≥ 1.160 resulted from Standard Agglutination Test (SAT) and titers ≥ 1.60 resulted from 2ME Test during sampling time were considered as positive. All the participants with seropositive results administrated with conventional

medication for Brucellosis with Riphampin and Doxycycline for a period of six weeks. Thereafter, three months later all the participants were subjected to sampling and the blood serum samples were seprated as mentioned above. The serum level of IL-23 was investigated by ELISA diagnostic kit (Biosource, UK).

2.3. Statistical Analysis

The recorded data were analyzed by Anova analytical method using SPSS software.

3. Results

The recorded data showed that the levels of IL-23 in the serum samples obtained from group A (143.64 Pg/ml) significantly (P<0.001) increased compared with this value in group B (23.14 Pg/ml). Based on the recorded data in the forms completed by all the participants at the day 0 of the experiment, 44 out of 186 individuals in group A, had experienced Brucellosis attack 2-3 times in spite of receiving medical prescriptions. A hypothesis about the possible immune system disorders in these participants lead us to did the re-sampling following drug administration.

The repeated blood sampling and serum analysis of these repeated samples showed that in spite of significant decrease in IL-23 level in 132 participants, in the rest of participants (n=54) higher levels of IL-23 were observed in peripheral blood. The results showed that 21 samples out of these 54 participants with higher level of IL-23 were belonged to the 44 people who had previous experience of Brucellosis affliction. The average level of IL-23 in these 21 participants was equal to 17.42 Pg/ml which was significantly different from that of control group (B) during second sampling with 26.51 Pg/ml (P<0.001). Typical symptoms of Brucellosis were recorded again in these 21 participants. The Results of SAT titer was higher than 1.160 in these participants. This amount was not significantly different from what obtained from post-chemotherapy samples.

4. Discussion

IL-23 is a cytokine which belongs to IL-6 super family (14). The pivotal role of IL-23 in inflammations due to intracellular infections which are associated with Th17, has been recognized (15). IL-23 which is very similar to IL-12 in terms of structure regulates enormous inherent and acquired immunological processes (16). IL-23 can increase the influential activity of T cells and at the same time the capability of antigen-providing cells including macrophages (17). Some studies have shown that in certain cases when immune system of body is stimulated by general stimulators of cell-mediated immune responses (CMI), IL-23 enforces its pathological effects through IL-17. Since the conducted studies have indicated that IL-23 receivers are mostly expressed on Th17 cells it is more than likely that IL-23 has a critical role in differentiation and preservation of Th17 cells phenotypes. Although this important role has been demonstrated by several studies, the mechanism through which intracellular infections including Brucellosis pathogen increase IL-23 production has remained unknown (14). Increase in population and activity of Th17 cells results into increase of IL-17 secretion from these cells. Thus, we can conclude that increase of IL-23 makes immune responses paths more active and as a consequence there will be an increase in gene expression and production of pivotal cytokine for cell-mediated immune responses i.e. IFN-Y. Several studies have shown that if IL-23 subunits are targeted to genetic destruction, certain disturbances may occur either in sensitivity increasing responses or in immune responses paths associated with self-immune diseases (18, 19). Yet, there are other studies which indicate the stimulating effect of IL-23 on transformation of Th17 cells function into cells with high infiltration power (14). On the other hand, some evidences are available which show that mice with mutant IL-23 producing gene suffer from serious problems associated with inflammation. It has been reported that IL-23 plays a major role in responding to bacterial infections.

Protective immune responses against infections which are directed through IL-23 axis are usually associated with cytokine axis of IL-23/IL-17. The activity of this axis leads to an increase in macrophagic activity and accumulation of neutrophil. The present study shows that when IL-23 production decreases in patients with Brucellosis the treatment process through administration of conventional drugs may become disrupted and ineffective. Additionally, measuring IL-23 in the second phase verified the significant role of IL-23 in Brucellosis treatment. Some impairments of IL-23 production path are observable in the case of the subjects having previous records of affliction with Brucellosis. Understanding whether this defect is related to IL-23 production path itself or other factors may have influence on it, requires other specific researches devoting to this matter. Presently, some studies are being conducting focusing on investigation of genetic sequence and level of this cytokine's gene expression, and the resultant findings will be reported in future works.

Authors' Contribution

Study concept and design: A. I. R. and A. M. R.
Acquisition of data: M. G. A. and M. A. J.
Analysis and interpretation of data: H. T. M. and M. G. A. R.
Drafting of the manuscript: A. A. A. and H. A. T.
Critical revision of the manuscript for important intellectual content: S. A. and A. J. O.
Statistical analysis: A. I. R. and A. J. O.
Administrative, technical, and material support: H. T. M.

Ethics

The ethics committee of the Al-Mustaqbal University College, Babylon, Iraq approved the study protocol

Conflict of Interest

The authors declare that they have no conflict of interest.

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