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Immunogenetic Study on Acute and Chronic Brucellosis in Diyala province

A Thesis

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By

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Chapter One

1.1. Introduction:

Brucellosis is one of major bacterial zoonotic infectious diseases. It is worldwide distributed; however, it is rare in most industrialized countries and more common in low-income and middle-income countries (Franc *et al*., 2018). Brucellosis is endemic in Iraq and Middle East countries has the highest reported prevalence of brucellosis in the world (Daood *et al.,* 2020). Actually it is one of the neglected diseases in these areas. Brucella species are gram-negative, nonmotile, non-encapsulated, and facultative intracellular bacteria that can cause acute and chronic zoonotic disease in humans (Sofian *et al.,* 2016). The genus *Brucella* consisted of six recognized species, grouped according to their primary host preferences, that is, *B. abortus* (cattle), *B. melitensis* (sheep and goats), *B. suis* (pigs), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (wood desert rats). Recent isolates from human (*B. inopinata*), aquatic mammals (*B. pinnipedialis and B. ceti*), and a common vole (*B. microti*) are recognized as new species, bringing the current number to 10 species in the genus (Adem *et al.,* 2021). Domestic and wild animals are reservoirs of the bacteria, which are usually transmitted to humans through the consumption of contaminated unpasteurized dairy products, direct contact with infected animals, and inhalation of infected aerosols (Baldi and Giambartolomei, 2013). In human it mostly affect bones and joints, largely reported in all ages and sexes in high-risk areas (Adetunji *et al*., 2019).

Clinically, there were a wide range of clinical manifestations such as undulant fever, night sweat, malaise, insomnia, arthralgia, nervousness, asthenia, depression (Jafari *et al*., 2015) and sexual impotence (Khan and Zahoor, 2018). Human brucellosis is also known for multiple organ involvement causing encephalitis, meningitis, endocarditis, arthritis,

orchitis, and prostatitis (Samaha *et al*., 2008). Although non-specific and often misleading clinical presentation of active brucellosis has made it a diagnostic puzzle; however, several laboratory tests had been developed for the diagnosis of brucellosis, mostly relay on anti-brucella IgM and IgG antibodies as well as the molecular methods based on PCR techniques (Alişkan, 2008; Jindan *et al*., 2019; Tekle *et al*., 2019; Dal *et al*., 2019).

Brucella species survive within a variety of cells, including macrophages, and spread in mononuclear phagocytes to reticuloendothelial sites (Rasouli *et al.*,2013). Both cell-mediated and humoral immunity are responsible for the clearance of brucella infection (Orozco *et al.,* 2003; Eskandari-Nasab *et al.,* 2013). Host protection against *Brucella species* primarily depends on cell-mediated immunity, involving mainly activated antigen-presenting cells (macrophages, dendritic cells) and CD4+ and CD8+ T-lymphocytes (Eskandari-Nasab *et al.,* 2013). Many Studies have shown that the changes of T lymphocyte are crucial to the interpretation of the clinicopathological features of brucellosis in the process of chronic infection and recurrence (Velasquez *et al.,* 2012; Skendros and Mitroulis, 2012). There are three mechanisms of acquired immunity in Brucella infection: (1) the antimicrobial effect of macrophage induced by interferon-gamma secreted by CD4+, CD8+, γ, and β T lymphocyte (Durward *et al.,* 2012) ; (2) the hosts eliminate the macrophages infected by Brucella through the cytotoxic effect of CD8+, γ, and β T lymphocytes (Durward-Diioia *et al.,* 2015); and (3) the modulating effect of the antibody can enhance the phagocytosis of macrophages (Baldwin and Goenka, 2006). Activated CD8+ T lymphocytes and CD4+ Th1 type immune responses play an important role in the scavenging of intracellular pathogens. *Treg* has the function of inhibiting the proliferation of T lymphocyte and eliminating the pathogen. The changes in the number and function of Treg inhibit the

immune function of the host, which may be one of the mechanisms that leads brucella infection progresses into chronicity (Wang *et al.,* 2014).

Because the cytokine production profile varies in the different stages of brucellosis, the serum levels of cytokines can be used as candidate prognostic markers for human brucellosis. Assessing cytokine levels in patients with acute and chronic brucellosis is not only useful for detecting the immune response, but can also be indicative of the severity of brucellosis (Lin *et al.,* 2020). After entering the human body, Brucella likely activates immune cells and promotes the production of cytokines by T lymphocytes. It found that IL-17, IFN-γ, and TNF-α levels were higher in patients with brucellosis than healthy controls, indicating a strong immune response was occurring. Indeed, previous studies have shown Brucella is effectively cleared through the secretion of IFN-γ, IL-2, and TNF-α by T helper (Th) 1 cells (Giambartolomei *et al.,* 2002; Celli, 2006). Th1 cells play a central role in immunity to brucellosis, beside the strong cooperative role for Th1 and Th17 cells in immunity to brucellosis which is more evident during acute and relapse phases of brucellosis (Rahmanpour *et al.*, 2019).

Interleukine-18 plays a protective role against brucellosis (Pasquali *et al.,* 2001) . It has been found that Caspase-1-related inflammasomes are sufficiently activated to induce the secretion of cytokines, such as IFN-γ and IL-18, which are significantly increases to induce cellular immune response (Karaca *et al.,* 2019). On the other hand, During Chronic brucellosis reduces lymphocyte proliferation and Th1 cytokine secretion, enhances IL- 5 and TGF-β production, affirming that the immune responses plays a crucial part in the progression and development of chronic diseases (Ghaznavi *et al.*, 2017).

Production and release of cytokines rely mostly on human genetic factors, so variations in the regulatory sequences of the cytokine genes can greatly affect the cytokine balance. It is extensively documented the role of cytokines in protection and amelioration of the clinical course of brucellosis (Elfaki *et al*., 2015; Kayhan *et al*., 2016; Lopez-Santiago *et al*., 2019). Some of these cytokines were remain in the body for longer time after treatment (Rasouli *et al.,* 2013; Xu *et al*., 2019) .The connection between cytokine gene polymorphisms and brucellosis disease status was studied in various populations with variable results (Bravo *et al*.,2003; Karaoglan *et al*., 2009; Eskandari-Nasab *et al*., 2013). A few studies report an association between cytokine gene polymorphism and development of brucellosis (Bravo *et al*., 2003; Rezazadeh *et al*., 2006; Rasouli *et al*., 2007; Karaoglan *et al*., 2009). Moreover, the serum levels of IL-6, IFN-gamma and TNF-α were significantly higher in brucellosis patients, but IL-1β, TGF-β1, IL-2, IL-4 and IL-8 levels are insignificantly higher in patient. There was a positive correlation between IFN-γ, TNF-alpha and IL-6 levels with CRP levels. IL-6, IFN- γ and TNF-alpha levels were significantly lowered post-treatment (Lin *et al*., 2020).

A growing number of studies report higher prevalence of single nucleotide polymorphisms (SNPs) in the cytokine-encoding genes of patients with brucellosis. These SNPs are possibly the agents responsible for susceptibility to brucellosis and can be important in the clinical course and prognosis of the disease (Davoudi *et al.,* 2006; Zafari *et al.,* 2020). It has been found that several potentially functional single nucleotide polymorphisms (SNPs) have been identified in the human IFN-γ R1 gene promoter and the protective role of TNF-α-857C/T and TNF-α-308 G/A polymorphism against human brucellosis (Ismael *et al*., 2016; Ismael *et al*. 2018; Naseri *et al*., 2019). In regard to IL-17 which is one of the most important inflammatory cytokines that stimulate immunity responses in

humans infected with brucella species and that the induction of SNPs in the IL-17 gene was found to be associated with resistance to brucella infection (Rasouli *et al*., 2013; Keramat *et al*., 2019). For the best of our knowledge, no previous such study on the effect of interleukin genes polymorphism on the brucella infection in Iraq.

1.2. Aims of Study:

The current study aimed to:

1. Exploration of the anti-brucella IgM and IgG among patients clinically diagnosed as brucellosis.
2. Detection of certain inflammatory cell markers such as CD3, CD4, and CD8 using ELISA techniques.
3. Detection of certain proinflammatory cytokines like IL-17, IL-18 and IFN-γ and their titers by ELISA techniques.
4. Detection of interleukin 17, IL-18 and IFN-γ genes polymorphism using PCR techniques.
5. Searching for all possible association of these markers that may promote accurate diagnosis of brucellosis.

الخلاصة

يحدث داء البروسيلات بسبب بكتريا من جنس البروسيلا *Brucella* والتي هي بكتريا صغيرة سالبة لصبغة جرام غير متحركة وغير مكونة للابواغ وهي عصيات مكورة هوائية اختيارية توجد داخل الخلايا قادرة على غزو الخلايا الظهارية والبانيات التغذوية المشيمية والخلايا التغصنية اضافة الى البلاعم الكبيرة. توجد أربعة أنواع منها تسبب الاصابة للبشر وهي: *B.abortus* و *B.canis* و*B.mellitunsis* و *B.suis*. تشمل الأعراض الحمى المتموجة والتعرق الغزير وآلام المفاصل والعضلات. يعد داء البروسيلات أحد أكثر الأمراض حيوانية المصدر شيوعًا في جميع أنحاء العالم مع وجوده كمرض متوطن في بعض المناطق في الشرق الأوسط وآسيا الوسطى وأفريقيا وأمريكا الوسطى والجنوبية. تحدث الاصابة في الإنسان بشكل اساسي من ملامسة الماشية المصابة أو استهلاك منتجات حيوانية ملوثة و تعتبر الماشية والحيوانات المجترة الصغيرة مضائف خازنة رئيسية للمرض.

استهدفت الدراسة الحالية تحديد أبرز المظاهر المناعية الخلوية والخلطية لداء البروسيلا الحاد والمزمن وهي (CD3، CD4، CD8، IL-17، IL-18، IFN-γ) واستكشاف تعدد اشكال جينية معينة للمضيف والتي قد تؤثر على حساسية وضراوة داء البروسيلات.

أجريت هذه الدراسة المقطعية العرضية في مدينة بعقوبة – محافظة ديالى للفترة من شهر شباط 2020 إلى شهر حزيران 2021 وشملت 55 مريضاً يشتبه سريرياً بإصابتهم بداء البروسيلات حيث كانت نتائجهم إيجابية اساسا لاختبار تراص روز بنغال. تراوحت أعمار المرضى بين (12 و 65) عامًا (11 من الذكور و 44 من الاناث) تم اختيارهم من مستشفى بعقوبة التعليمي ومستشفى البتول التعليمي للولادة والأطفال بالإضافة الى 35 شخصا من الاصحاء كمجموعة سيطرة (16 من الذكور و 19 من الاناث) تراوحت أعمارهم بين (14و65) سنة. تمت الموافقة على اجراء هذه الدراسة