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# Effectiveness of Brucellosis on Polymorphisms of IL-1B and IL-10 Concentrations Among Iraqi Patients

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**ABSTRACT**

**In this study, (60) blood samples were taken from patients infected with brucellosis, and (60) samples were taken from healthy individual as a control group. The patients were visiting Emam Ali Hospital for Fever in Baghdad city, during the period from 1st February to 1st November 2023. The results of gender distribution showed that among patients infected with Brucellosis, 30 (50.0%) were males and 30 (50%) were females, while among the control group, 31(51.7%) were males and 29(48.3%) were females. Also the distribution of infections according residency were matched between urban and rural locations 30(50.0%) for both. While the distribution of Brucellosis among age groups showed that the highest incidence of infection was in the age groups (40-50) years and (17-29) years 22(36.7%) for each, followed by the age group (30-39) years 16(26.7%). The mean levels of anti Brucella antibodies IgM, IgG, IL-10, IL-1B in patients with brucellosis were (0.11±0.17), (0.09±0.15), (5.62±1.99), (4.92±1.76) respectively compared to their mean levels in the control group (2.01±1.17), (1.33±0.59), (60.68±26.81), (22.85±9.17) respectively with highly significant differences (p<0.01). The ROC test showed that the Sensitivity of Brucella IgM concentration was100% and Specificity 100% in Cut off >0.84. Also the Sensitivity of Brucella IgG concentration was 100% and Specificity 100% in Cut off >0.33. Also the Sensitivity of IL-10 concentration was 100% and Specificity 100% in Cut off >18.04, and the Sensitivity of IL-1B concentration was 100% and Specificity 100% in Cut off >9.08. There were mutations occurred with IL-1B gene ID 3553 in SNPs, rs1143627. The variation of wild TT was changed to CC, CC, TC, TC, CC, AG, CC, TC in 8 cases out of 11 in comparison with the control group, and the mutation occurred with IL-1B gene ID 3553 in SNPs, rs1143627. The variation of wild TT was changed to CC, CC, TC, TC, CC, AG, CC, TC in 8 cases out of 11 in comparison with the control group.**

***Keywords***- Brucellosis, Polymorphisms, IL-1B, IL-10**.**

## I. INTRODUCTION

Brucellosis is a common zoonotic disease that is usually detected in animals and humans [1]. Although eradication efforts are continuously carried out, the problem of brucellosis exists in endemic areas in which brucellosis is not an important health problem till now [2]. The clinical manifestations of Brucellosis are wide and usually last from few days to many years. Brucellosis in humans seldom causes death but is generally devastating [3]. The disease has different transmission routes such as skin, gastrointestinal tracts, respiratory tracts and mucus membrane in addition to the contacts with body fluid [4]. The consumption of uncooked meat and dairy product, are the main routes of transmission of brucellosis from animals to humans [5]. Misdiagnosis of brucellosis is frequent and may result in prolonged sickness and insufficient treatments [6]. In addition, the non-specificity of the disease symptoms makes its diagnosis difficult. The prevalent epidemiological situations of brucellosis in vulnerable animals (wildlife and livestocks) in a region or a country plays an essential role in selecting specific strategies in diagnostic tests [7]. Diagnostic tests may be applied for different goals, such as confirmation diagnosis, screening or prevalence studies as well as disease confirmation. In areas where brucellosis is eliminated [9], surveillance is required to ensure prevention of brucellosis reintroduction via importing infected animals or their products [8]. The validity of these tests, particularly in wildlife, is still a problem[10].

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There is an important role of IL-1β in different pain cases, such as the roles of the intracellular complexes, the inflammations that regulate IL-1β productions [11]. Evidences will be introduced showing the IL-1β importance in both pain induction and in pain maintenance in chronic conditions, e.g. following nerve injuries. Moreover, IL-1β involvement as a main mediator in the interactions between neurons and glia in pain conditions will be discussed [12]. The evidences introduced in the present review collectively showing the significance of IL-1β in human and animal pain conditions, indicate that IL-1β blockade can be regarded as a therapeutic opportunity [12]. , IL-10, a Th2 cytokine, is able to suppress the macrophage functions and raise the infection susceptibility [13]. The acute brucellosis in humans have also been described. In human brucellosis, there is little information about the precise role of IL-4 (a Th2 cytokine) and TNF-α [14]. The study aimed to detecti the polymorphism of IL-1B and IL-10 genes in Iraqi patients with brucellosis.

## II. MATERIALS AND METHODS

In this study, (60) blood samples were taken from patients infected with brucellosis, and (60) samples were taken from healthy individual as a control group. The patients were visiting Emam Ali Hospital for Fever in Baghdad city, during the period from 1st February to 1st November 2023. The concentrations of Brucella IgM and IgG were estimated by the enzyme immunoassay (EIA) technique, while the concentration of IL-10 and IL-1B was estimated by the sandwich enzyme-linked immune-sorbent assay technology. Molecular diagnosis was carried out by PCR technique, the primers used were: IL1B-F: TGTAAAACGACGGCCAGTCCTGGACTC CTCTCATTCATTCTAC, IL1B-R: CAGGAAACAGCTATGACCTCGAAGAGGTTTGGT ATCTG, IL10-F TGTAAAACGACGGCCAGTAAGTAAGGGACCTCC TATCC, IL10-R:CAGGAAACAGCTATGACAGAGCTC CTCCTTCTCTAAC. Gene sequence was done by signer sequencer.

***Statistical analysis:*** The SPSS-20 software program was used for statistical analysis of data, including the t-test. The ($P<0.05$) value was considered as significant.

## III. RESULTS

The demographic picture of gender distribution showed that among patients infected with Brucellosis, 30 (50.0%) were males and 30 (50%) were females, while among the control group, 31(51.7%) were males and 29(48.3%) were females. Also the distribution of infections according residency were matched between urban and rural locations 30(50.0%) for both. While the distribution of Brucellosis among age groups showed that the highest incidence of infection was in the age groups (40-50) years and (17-29) years 22(36.7%) for each, followed by the age group (30-39) years 16(26.7%), as shows in table (1).

**Table (1): Distribution of Study groups according to Demographic characteristics**

| Demographic characteristics | | Study groups | |
|---|---|---|---|
| Gander | | Control(n=60) | Patients(n=60) |
| Male | No. | 31 | 30 |
| | % | 51.7% | 50.0% |
| Female | No. | 29 | 30 |
| | % | 48.3% | 50.0% |
| Total | No. | 60 | 60 |
| | % | 100.0% | 100.0% |
| Residence | | Control | Patients |
| Urban | No. | 30 | 30 |
| | % | 50.0% | 50.0% |
| Rural | No. | 30 | 30 |
| | % | 50.0% | 50.0% |
| Total | No. | 60 | 60 |
| | % | 100.0% | 100.0% |
| Age groups(year) | | Control | Patients |
| (17-29) | No. | 25 | 22 |
| | % | 41.7% | 36.7% |
| (30-39) | No. | 23 | 16 |
| | % | 38.3% | 26.7% |

**Journal for Research in Applied Sciences and Biotechnology**
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| | | | |
|---|---|---|---|
| (40-50) | No. | 12 | 22 |
| | % | 20.0% | 36.7% |
| Total | No. | 60 | 60 |
| | % | 100.0% | 100.0% |

The mean levels of anti Brucella antibodies IgM and IgG in patients with brucellosis were (0.11±0.17), (0.09±0.15) respectively compared to their mean levels in the control group (2.01±1.17), (1.33±0.59) respectively with highly significant differences (p<0.01), as shown in table (2).

**Table (2): Distribution of brucella IgM and IgG among the study groups**

| | **Study groups** | **(Mean ±Std.)** | **Levine's Test(F)** | **P-Value** |
|---|---|---|---|---|
| Brucella IgM | Control | (2.01±1.17) | 57.224 | P=.000 P< .01(HS) |
| | Patients | (0.11±0.17) | | |
| Brucella IgG | Control | (1.33±0.59) | 64.822 | P=.000 P< .01(HS) |
| | Patients | (0.09±0.15) | | |

The mean levels of anti IL-10 and IL-1B in patients with brucellosis were (5.62±1.99), (4.92±1.76) respectively compared to their mean levels in the control group (60.68±26.81), (22.85±9.17) respectively with highly significant differences (p<0.01), as shown in table (3).

**Table (3): Distribution of IL-10 and IL-1B among the study groups**

| | Study groups | (Mean ±Std.) | Levine's Test(F) | P-Value |
|---|---|---|---|---|
| IL-10 | Control | (60.68±26.81) | 126.266 | P=.000 P< .01(HS) |
| | Patients | (5.62±1.99) | | |
| IL-1B | Control | (22.85±9.17) | 75.085 | P=.000 P< .01(HS) |
| | Patients | (4.92±1.76) | | |

Table 4 and figure 1 showed that with ROC test, the Sensitivity of Brucella IgM concentration was 100% and Specificity 100% in Cut off >0.84. Also the Sensitivity of Brucella IgG concentration was 100% and Specificity 100% in Cut off >0.33.

**Table (4): Receiver Operating Characteristic Curve analysis (ROC) of Brucella IgM and Brucella IgG**

| Variable(s) | Area | P-Value | Cut off | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Brocella IgM | 1.000 | .000 | >0.84 | 100% | 0.00 % |
| Brocella IgG | .988 | .000 | >0.33 | 100% | %53.3 |

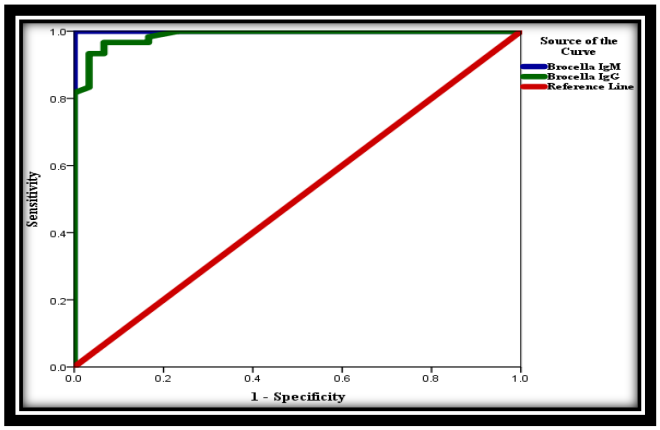


**Figure (1): Receiver Operating Characteristic Curve analysis (ROC) of Brucella IgM and Brucella IgG**

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Volume-2 Issue-6 || December 2023 || PP. 247-253

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Table 5 and figure 2 demonstrated that in ROC test, with Brucellosis, the Sensitivity of IL-10 concentration was 100% and Specificity 100% in Cut off >18.04. Also the Sensitivity of IL-1B concentration was 100% and Specificity 100% in Cut off >9.08.

**Table (5): Receiver Operating Characteristic Curve analysis (ROC) of IL-10 & IL-1B**

| Variable(s) | Area | P-Value | Cut off | Sensitivity | Specificity |
|---|---|---|---|---|---|
| IL-10 | 1.000 | .000 | >18.04 | 100% | 0.00 % |
| IL-1B | 1.000 | .000 | >9.08 | 100% | 0.00 % |

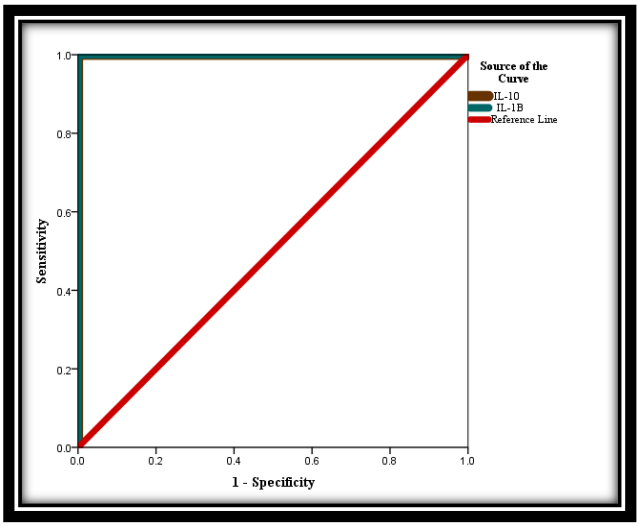


**Figure (2): Receiver Operating Characteristic Curve analysis (ROC) of IL-10 & IL-1B**

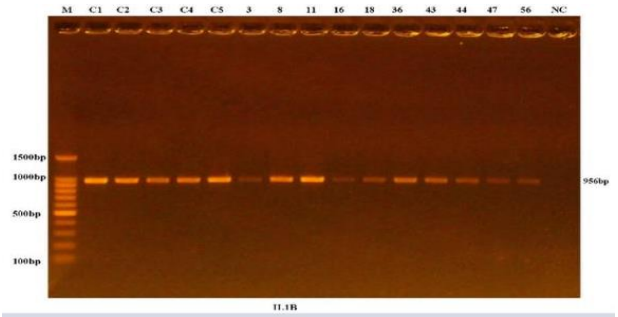


**Figure (3): Results of implication of IL-10 specific regions of Human sample species are fractionated in 1.5 agarose gel electrophoresis stained by Ethidum Bromide M:100bp ladder Markers. Lanes Cr-56 resemble 961 PCR products.**

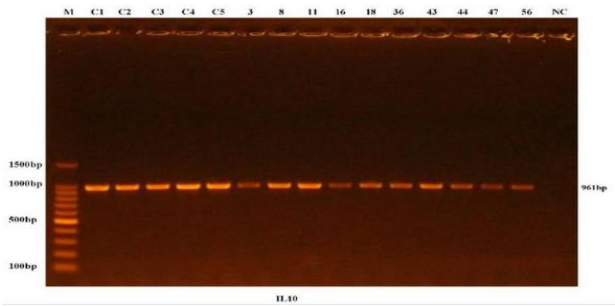


**Figure (4): Results of implication of IL-1B specific regions of Human sample species are fractionated in 1.5 agarose gel electrophoresis stained by Ethidum Bromide M:100bp ladder Markers. Lanes Cr-56 resemble 956 PCR products.**

Table (6) and Figure (5) showed that a mutation occurred with IL-1B gene ID 3553 in SNPs, rs1143627. The variation of wild TT was changed to CC, CC, TC, TC, CC, AG, CC, TC in 8 cases out of 11 in comparison with the control group.

**Table (6): Variation of wild SNPs of IL-1B gene ID 3553**

| IL1B GENE ID 3553 | | |
|---|---|---|
| **SNPs** | **rs16944** | **rs1143627** |
| **Wild** | **TT** | **CC** |

| **Variation** | **T>C** | **C>T** |
|---|---|---|
| **Samples** | | |
| 3 | TT | CC |
| 8 | CC | TT |
| 11 | CC | TT |
| 16 | TC | CT |
| 18 | TC | CC |
| 36 | CC | TT |
| 43 | CC | TT |

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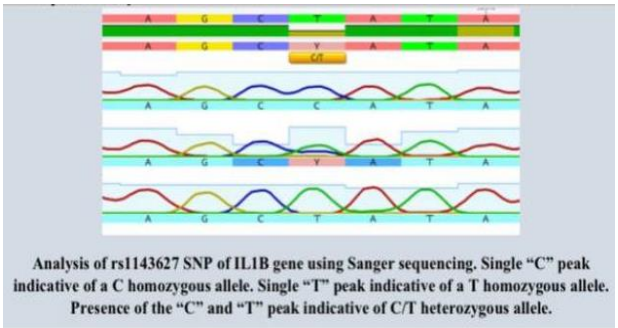
Volume-2 Issue-6 || December 2023 || PP. 247-253

https://doi.org/10.55544/jrasb.2.6.34

| | | |
|---|---|---|
| 44 | TC | CT |
| 47 | TT | CC |
| 58 | TT | CC |
| C1 | TC | CC |
| C2 | CC | TT |
| C3 | CC | TT |
| C4 | TC | CT |
| C5 | CC | TT |



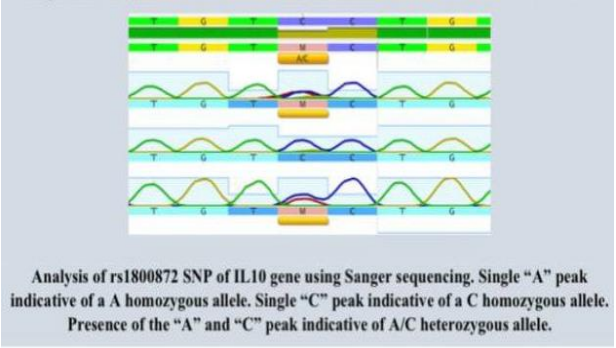
Analysis of rs1143627 SNP of IL1B gene using Sanger sequencing. Single "C" peak indicative of a C homozygous allele. Single "T" peak indicative of a T homozygous allele. Presence of the "C" and "T" peak indicative of C/T heterozygous allele.

**Figure (5): The sequence of IL-1B gene**

Table (7) and Figure (6) showed that a mutation occurred with IL-10 gene ID 3553 in SNPs, rs1143627. The variation of wild AA was changed to CC, CC, AC, TC, CC, AG, CC, AC CC, CC, CC, in comparison with the control group.

**Table (7): Variation of wild SNPs of IL-10 gene ID 3586**

| IL10 GENE ID 3586 | | |
|---|---|---|
| **SNPs** | **rs1800871** | **rs1800872** |
| **Wild** | **TT** | **AA** |
| **Variation** | **T>C** | **A>C** |
| **Samples** | | |
| 3 | CC | CC |
| 8 | CC | CC |
| 11 | TC | AC |
| 16 | CC | CC |
| 18 | TC | AC |
| 36 | CC | CC |
| 43 | CC | CC |
| 44 | CC | CC |
| 47 | CC | CC |
| 58 | CC | CC |
| C1 | CC | CC |
| C2 | TC | AC |
| C3 | CC | CC |
| C4 | CC | CC |
| C5 | CC | CC |



Analysis of rs1800872 SNP of IL10 gene using Sanger sequencing. Single "A" peak indicative of a A homozygous allele. Single "C" peak indicative of a C homozygous allele. Presence of the "A" and "C" peak indicative of A/C heterozygous allele.

**Figure (6): The sequence of IL-1B gene**

## IV. DISCUSSION

According to the results, the mean of anti Brucella IgM antibodies concentration (0.11±0.17) in Brucellosis patients was significantly higher than the control group, and this finding agreed with (Salmanzadeh, et al, 2020 and Yousaf, et al, 2021) who reported that there is a very noticeable increase in the concentrations of antibodies for those infected with acute Brucellosis, which confirms the increased capacity of the immune system to defend the body and the presence of white cells that fight the infection [15,16]. Also the mean of anti Brucella IgG antibodies concentration in Brucellosis patients was significantly higher than the control group, which was matched with (Guzmán-Bracho, et al, 2020) who reported that in the stage of chronic infection with Brucella, the immune system is occupied with the production of antibodies and defense cells, especially since there is no effective treatment that kills this bacteria that is transmitted through animals by drinking unsterilized milk, there will be a high rise and strong reaction of the immune system constantly [17]. The mean of IL-10 concentration in Brucellosis patients was significantly higher compared to the control group, a result that was matched with (Tang, et al, 2021) who confirmed that in acute brucellosis, the levels of IL-2, IL-10 & IFN-γ are significantly elevated in comparison with the healthy individuals [18]. Also, the mean of IL-1B concentration in Brucellosis patients was significantly higher than the control group. Sun, (2021) revealed that the Treg-related cytokines in patients with acute and chronic brucellosis were assessed to find their effect at various stages of brucellosis. The immunity of Treg cells is included in brucellosis chronicity and suggests the implication of Tregs in brucellosis prognosis. CTLA-4 and TGF-β1 can participate in Tregs-mediated immunosuppressions in the chronic phase of brucellosis [19]. A mutation occurred with IL-1B gene ID 3553 in SNPs, rs1143627. The variation of wild TT was changed to CC, CC, TC, TC, CC, AG, CC, TC in 8 cases out of 11 cases, (Demirdag, et al, 2003) reported that macrophages are recruited by interleukin-1β (IL-1β) and granulocyte-macrophage colony stimulating factor (GM-CSF) [20]. A mutation occurred with IL-10 gene ID 3553 in SNPs, rs1143627. The variation of wild AA was changed to CC,

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Volume-2 Issue-6 || December 2023 || PP. 247-253
https://doi.org/10.55544/jrasb.2.6.34

CC, AC, TC, CC, AG, CC, AC CC, CC, CC in comparison with the control group. (Jin, et al, 2021) reported that for position − 1082 G/A of IL-10, there was an allele comparison (A versus G), homozygote comparisons (AA versus GG), recessive model (AA versus AG/GG), and dominant models (AA/AG versus GG). For position − 819 C/T of IL-10, there was an allele comparison (T versus C), homozygote comparisons (TT versus CC), recessive models (TT versus TC/ CC), and dominant models (TT/TC versus CC) [21,22].

## V. CONCLUSIONS

According to the results, there were mutations occurred with IL-1B gene ID 3553 in SNPs, rs1143627. The variation of wild TT was changed to CC, CC, TC, TC, CC, AG, CC, TC in 8 cases out of 11 cases, and a mutation occurred with IL-10 gene ID 3553 in SNPs, rs1143627. The variation of wild AA was changed to CC, CC, AC, TC, CC, AG, CC, AC CC, CC, CC in comparison with the control group.

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**ISSN: 2583-4053**

Volume-2 Issue-6 || December 2023 || PP. 247-253

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