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Original Article

Serum Adenosine Deaminase Activity and C-Reactive Protein Levels in Patients with Brucellosis

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ABSTRACT

Background and Objective: **Brucellosis is a main transmittable zoonotic disease, which is endemic, and a common health burden in Iran. Adenosine deaminase (ADA) is an essential enzyme which is involved in purine metabolism and its role in immune system is very important. The aim of this study was to determine serum changes of ADA and C-Reactive Protein (CRP) levels in patients with brucellosis.**

Patients and Methods: **The study was a case-control one on 36 patients and 36 controls. The serum level of ADA and quantitative CRP was measured in both patients and controls. We also measured the Wright, Coomb's Wright and 2-mecapto ethanol (2ME) in two participants groups. Statistical analysis was performed using SPSS for windows Version 11.5**

Results: **ADA serum level in patients group showed a significant difference compared to control group (31.6425.1± vs. 13.973.9±, *P*<0.0001). Quantitative CRP level in patients group was higher than control group significantly (25 ±20.7 vs 6.94.4± , *P*<0.0001). There was a correlation between level of serum ADA and serum CRP in patients significantly (*P*<0.004) while there was no correlation between Wright, Coom's Wright, and 2ME with serum ADA and CRP levels (P=NS).**

Conclusion: **This finding shows the serum level of ADA and CRP are two important parameters in diagnosis, treatment of brucellosis with the considering of the clinical manifestations and other paraclinic findings. However it is advisable to perform more studies.**

Keywords: Brucellosis, Adenosine deaminase, C Reactive Protein

Introduction

Brucellosis is one of the most important transmittable diseases between humans and animals in Iran that is specially considered from the economic and general health point of view. Brucellosis in human is an indicator for the dissemination of this disease among the animals. Usually, direct contact with the infected animals or their products cause the

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incidence of infection in human.

This disease is endemic and prevalent in Iran and the determination of its incidence and appearing is hard due to the lack of perfect reports. The immunity response against the *Brucella* is by Th1 and Th2 lymphocyte cells and the ratio of Th1/Th2 shows the sensitivity or resistance in front of the infection produced by *Brucella* (1).

Some studies imply the reduction of T cells proliferation and Th1 cytokines in brucellosis (1, 2). Adenosine D-aminase (ADA) is a main enzyme for discrimination and T proliferation of lymphocytes and macrophage monocyte system (2). ADA participates in pourin metabolism and has an obvious role in the mechanisms of immunity system. Although the increase of ADA activity is usually observed in tuberculosis but also can be obvious in the other infectious or non-infectious diseases such as typhoid fever, sarcoidosis, and acute lymphoblastic leukemia (3-6).

In ADA genetic defect that is a recessive autosomal disease and leads to the functional disorder of T and B lymphocyte cells we confront with the reduction of ADA activity (7). *Brucella* is a intracellular gram negative bacillus or cocobacillus and cellular immunity has the main role in controlling it.

It seems that the ADA serum activity increases in the current of brucellosis and measuring it may help us in the diagnosis and the follow up of disease (1).

In this study the ADA serum activity of subjects wre measured and compared in two groups of patients affected by brucellosis and controls. The secondary aim of this study was the evaluation of quantitative CRP serum level in two groups of controls and patients.

Material and Methods

A total number of 36 patients and 36 control subjects participated in this case-control study. The patients group were chosen out of out-patients or bedridden affected by brucellosis and had been referred to Imam Khomeini Hospital of Tehran University of Medical Science, Iran.

The patient group in addition to having clinical demonstrations had the Wright test $\geq$ 1/60 or the Coomb›s test $\geq$ 1/80 and aged between 12 and 65 years. Control group in addition to not having the background disease and negative serology for brucellosis were equal with patients group with regard to the age and sex and were chosen from the natives.

In addition to the clinical demography and medical examination a 6 ml sample volume of venous blood were taken from all patients. Wright, Coomb›s Wright, 2 ME, ADA and quantitative CRP serum tests were done on all of the subjects. Measured of ADA (Turbidometric method ,Biosystem Spain Company Kit) and quantitative CRP (Turbidometric method, Biosystem Spain serum levels was done by alcion -300 auto –analyzer (Abott , USA) in central laboratory of Imam Khomeini Hospital. Normal range for serum ADA was 15-25 IU/L and for quantitative CRP was <10 mg/L.

All of the data analysis was done by using SPSS software (version 11.5) under Windows based system. The implemented statistical tests were consisting of non parametric Kolmogrov-Smirnov test, non normal distribution t-test and Mean-Whitney U test. X^2 test was used for comparing the relation of qualitative variables. For quantitative variables the variance, mean, standard deviation and some parameters such as P-Value were calculated. All of the results were evaluated and considered duplicate. The accepted P values were lower or equal than 0.05.

Results

The mean age of participated patients in this study was 42.13 ± 16.77 years old and for control group was 42.38 ± 16.67. Each of these two groups were consisted of 25 males (69.4%) and 11 females (30.6%). From educational point, 44.4% were uneducated, 16.7% up to primary school, 13.9% up to guidance school, 16.7% up to high school and 8.3% had a university degree. From the point of profession in patient group the most percent of them were farmer, housekeeper and animal husbandry.

In patient group 66.7% were rustic and 33.3% were townsman. Among the patient group 63.9 had acute disease (2 month), 33.3% had sub-acute disease (2-12 month) and 2.8% had chronic disease (above 12 month).

61.1% of patients had not any brucellosis precedent, 25% had the precedent of one time, 11.1% had the precedent of two times and 2.8% had the precedence of three times to be affected by brucellosis.

In patient group, 94.4% had the precedent of using the local dairy products and 5.6% had not such a precedent. 77.8% had the precedent of contacting with animals and their products (except dairy products). The most prevalent sign and symptom of brucellosis was fever with 88.9% followed by anorexia, back

pain, weight loss, general body pain, chills, sweats, headache, fatigue, arthralgia, and nausea.

The minimum ADA activity in control group was 7 IU/L and the maximum of it was 22 IU/L with the mean of 13.9 IU/L (13.9 ± 3.9). The minimum ADS activity in patient group was 11 IU/L and the maximum of it was 132 IU/L with the mean of 31.64 IU/L (31.64 ± 25.1). Comparing of these groups with the mean ADA activity of 31.64 ± 25.1 IU/L for patients and 13.9 ± 3.9 IU/L for control had a valid difference (P<0.0001) (Table 1).

Table 1: Serum levels of ADA activity in patients and control groups

	Patients	Controls	*P* value
All	31.64 ± 25.1	13.9 ± 3.9	< 0.0001
Male	34.9 ± 25.9	14.4 ± 4.1	< 0.001
Female	24.2 ± 4.8	13 ± 3.4	< 0.001

ADA: adenosine deaminase activity

The minimum serum quantitative CRP in control group was 0 mg/L the maximum of it was 15.6 mg/L with the mean of 6.9 mg/L (6.9 ± 4.4). The minimum serum of quantitative CRP in patient group was 0.8 mg/L and the maximum of it was 80.8 mg/L with the mean of 25 mg/L (25 ± 20.7). Comparing of these groups with the mean serum CRP of 25 ± 29.7 mg/L for patients and 6.9±4.4 mg/L for control had a valid difference (P<0.0001) (Table 2).

Table 2: Serum levels of quantitative CRP in patients and control groups

	Patients	Controls	*P* value
All	25 ± 20.7	6.9 ± 4.4	< 0.0001
Male	27.3 ± 18.5	6.3 ± 3.7	< 0.001
Female	19.7 ± 25.1	8.6 ± 5.5	< 0.166

CRP: C-Reactive Protein

A valid difference was found between serum level of quantitative CRP and serum ADA activity (P=0.004) among the patients while no meaningful difference was seen in control group (P=0.328). There was no valid statistical relation between the titers of Wright, Commb ́s Wright and 2ME tests with serum levels of ADA and quantitative CRP.

Discussion

Brucellosis is a common disease between human and animals. The prevalence of brucellosis is high in Iran as an endemic region for this disease. Brucella is an intracellular gram negative bacterium that can be alive in reticuloendotelial cells system (8) comprising that the disease period has been chronic and sometimes shows the inability of antibiotics in proper cure of disease.

The protection against the intracellular bacteria depends on the function of T-lymphocytes and cytokines. ADA is a basic component for the maturity and operation of lymphocytes that exist in serum and most of the tissues especially in lymphatic tissues. Meanwhile the activity of T cells, ADA adjusts the activity of cytokines in cellular level. In this case, IL-2 and IL-12 have act as up – regulation and IL-4 plays the role of down –regulation (8-10).

The increase of serum levels of ADA has been reported in various diseases such as those are infectious, malignant and hepatic ones. This also occurs in brucellosis (1-3). In our study the difference of serum ADA in two groups of patients and controls was valid from the statistical point.

In the study of Viciana *et al*. on 67 patients affec-ted by brucellosis the serum activity of ADA was considered before the period of treatment and respectively for one, three and six month after that. The result of this study showed that the serum level of ADA was higher in patient group than control one (1).

In another study in Turkey, the indicator of ADA serum level in patients obviously was higher than controls (10). The value of ADA serum level was 27.5±9.3 in control group and 43.45 ± 24.19 in patients with $P<0.01$. The results of these studies are identical to our results. In those studies, it is mentioned that the ADA serum level has not a meaningful relation with the Brucella agglutination (10-15). In previous study it has been implied that it is impossible to distinguish between acute and chronic diseases based on ADA serum level (1;10) but as the number of chronic patients in our study was low, it is not possible to justify based on this subject and require to study with the enough number of cases.

In another study it has been also investigated on the serum quantitative CRP and reported that there was no valid difference between the serum level of ADA and CRP (10), while in our study there was a valid statistical relation between them.

It is reported that in acute phase of disease except in the case of IL-4 the other cytokines with ESR and CRP had a higher mean in comparison with the end of treatment and control group ($P<0.005$ in comparison with control group and $P<0.001$ compared with the end of treatment) (12). In addition, there was a meaningful and positive correlation between the CRP level and ESR value with the INF-δ and TNF-α level (12). Finally there was no meaningful difference between the INF-δ and TNF, CRP and ESR levels in control and patient groups. Consequently the researcher implied that TNF-α and INF-δ besides the ESR plus CRP had a close assistance with the phlogistic activity and suggested that these agents can be used as a factor for investigating the brucellosis disease (12). The results of these studies are confirming to those which are evaluating the increase of CRP in patient group in comparison with control one (11, 12, 16).

As we know, despite the clinical response of patient to treatment, the serum level of antibodies against the Brucella can be remained for two years of even higher. Therefore using these factors as a marker for following the disease is not recommended and not reducing the related titer antibodies is not indicator for non-response to the treatment (11,13,17).

Similarly to other studies, our results show that the ADA serum activity and quantitative CRP in the patients affected by brucellosis is obviously increased in comparison with healthy persons.

Conclusion

Consequently using the ADA serum level and quantitative CRP might be marker for the diagnosis and following the treatment process. More studies are suggested in this way. Considering that ADA has ADA1 and ADA2 enzymes, the evaluation of the type of these enzymes is suggested in another study.

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