JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Comparison of serum level of 25(OH) vitamin D3 in brucellosis patients with healthy persons in Hamadan, west of Iran

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Abstract

Introduction: The human immune system including phagocytosis, has anessential role in pathogenesis, relapse and improvement of infectious diseases. The immune cells have vitamin D receptor, and vitamin D deficiency causes impaired immune system, specifically macrophages. The aim of study was to compare serum levelof 25-hydroxyvitamin D3 (25-OH- VitD3) among the patients with acute brucellosis and relapsed brucellosis with healthy individuals .

Methodology: In this case-control study, 92 patients with acute brucellosis, 92 cases with relapsed brucellosis and 107 healthy persons who referred to Sina hospital and Imam Khomeini clinic were enrolled, and all groups were matched based on age, gender and habitat. The study was done from March 2016 to June 2017. The serum levels of 25-OH- VitD3 were measured based on the Enzyme Linked Fluorescent Assay (ELFA) technology, Vidassystem (France, Biomerieux Kit). The data were analyzed by using SPSS version 16 software.

Results: The mean serum levels of 25-OH- VitD3 in acute brucellosis, relapsed brucellosis and healthy persons were 22.55 ± 15.72, 26.82 ± 20.78, 24.44 ± 17.29, respectively .In addition, the mean serum levels of 25-OH- VitD3 by male gender in acute brucellosis, relapsed brucellosis and healthy control groups were 20.35 ± 13.10, 24.88 ± 20.89 and 22.52 ± 13.79, respectively. However, there was no statistically significant difference among three groups ($P = 0.275$).

Conclusions: According to the results, the prevalence of vitamin D deficiency was high in patients with acute or relapsed brucellosis and also healthy persons; however, there was no meaningful difference among three groups and between the patients and healthy persons.

Key words: acute; brucellosis; relapse; 25-hydroxyvitamin D3; Iran.

J Infect Dev Ctries 2018; 12(6):448-453. doi:10.3855/jidc.9731

(Received 01 September 2017 – Accepted 02 April 2018)

Introduction

Brucellosis is a bacterial zoonosis which transmitted to human from animals directly or indirectly. Mediterranean countries, margins of Persian Gulf, India and South America are endemic areas for brucellosis [1-3]. Human brucellosis is endemic in Iran that is in fourth place in the world. Incidence rate of brucellosis is high in Hamadan province [4,5].

Brucella spp. is an intracellular microorganism, so cellular immunity has a destructive role in brucellosis. It estimates that approximately 1 billion people in the world have moderate to severe vitamin D deficiency. On the basis of multiple studies, more than 50% of postmenopausal women, that receiving therapy for osteoporosis, have insufficientserum levels of 25-OH-VitD3 [6,7]. Twenty five-hydroxyvitamin D3 (25-OH-VitD3) stimulates osteogenic activity and regulates calcium metabolism, and also involves in host defense against microbial or inflammatory invasions. In addition, low serum levels of vitamin D_3 are associated with osteoporosis and elevated risk of bone fractures. Moreover, 25-OH- VitD3 induces the innate immune system and modulates B and T lymphocytes functions, and also influences inflammatory responses in inflammatory diseases. The importance of vitamin D and vitamin D receptor (VDR) on the regulation of immune system was suppoted in some studies. The hormonal form of 25-OH- VitD3 balanced anti-microbial peptides and increase clearance of bacteria in immune system cells [8-10]. Immune cells have receptors for 25-hydroxyvitamin D3 (25-OH- VitD3), and patients with vitamin D deficiency, have disturbances in immune system, specifically in macrophages [1-3].

Therefore, according to the relationship between 25-OH- VitD3and immune system [6-10], this study was conducted to compare serum levels of 25-OH-VitD3 in brucellosis patients with healthy individuals.

Methodology

Study design

In a case-control study, 92 patients with acute brucellosis, 92 cases with relapsed brucellosis and 107 healthy persons who referred to Sina hospital or Imam Khomeini clinic in Hamadan were enrolled, and all groups were matched based on age, gender and habitat. Inclusion criteria were age above 18 years, no history of vitamin D supplement therapy during recent 3 months,and without history of a disease affecting serum level of 25(OH) vitamin D3. The study protocol was compatible with the Declaration of Helsinki and approved by Research Ethics Committee of Hamadan University of Medical Sciences. The study was done from March 2016 to June 2017. All participants signed an informed consent before entering into the study. Acute brucellosis is defined as a patient who has clinical manifestations compatible with brucellosis,up to 8 weeks and positive serology (Wright >1/80 and 2ME > 1/40) or positive blood culture for Brucella [3,11].

Relapsed brucellosis is defined as a patient by reappearance of clinical signs and symptoms and positive serology with or without positive blood culture for Brucella within 6 months after discontinuation of therapy up to 2 years [2,3].

Instrument

After enrollment, 5cc blood sample from each participant was taken and transferred to Arad laboratory in Hamadan, Iran. The serum level of 25-OH- VitD3 was measured by measured based on the Enzyme Linked Fluorescent Assay (ELFA) technology, Vidas system (Biomerieux, Paris,France) [12].

In this study on the basis of Vidas reference, normal range of 25-OH- VitD3 was defined 30-100 ng/mL. Range of 21-29 ng/mL (52-72 nmol/L) serum level of 25(OH) vitamin D3 was determined as vitamin D insufficiency, and serum level lower than 20ng/mL (50 nmol/L) was marked vitamin D deficiency [12-14]. The preferred level for vitamin D (25-OH) by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L) [14-15].

Statistical analysis

All statistical analyses were done by using SPSS, version 16 (SPSS, Chicago, Illinois). To compare variables, Chi-Square, t test and unilateral variance were used. All hypotheses tests were 2-tailed with $P < 0.05$ considered significant. Data are presented as mean ± standard deviation (SD) for quantitative variables and frequency and percent for qualitative variables.

Results

Total number of studied persons was 291, including 92 patients with acute brucellosis, 92 patients with relapsed brucellosis, and 107 healthy persons (control group). The mean of age in acute brucellosis group was 44.22 ± 17.42 years (range: 14-82). The mean of age in relapsed brucellosis group was 47.74 ± 17.31 years (range: 15-86), and in control group, the mean of age was 44.06 ± 12.89 years (range: 14-79). All groups were matched on the basis of age and there was no significant statistical difference among three groups ($P = 0.199$).

Patients with acute brucellosis, relapsed brucellosis, and control group were matched on the basis of gender distribution ($P = 0.193$), and habitat too ($P = 0.746$). In acute brucellosis group, 51 male and 61 female, in relapsed group 60 male and 32 female, and in the control group 72 male and 35 female were studied.

The most observed complications in acute and relapsed brucellosis patients were bilateral sacroileitis (26.9%), spondylodiscitis (17.4%), spondylitis (12.6%), unilateral sacroileitis and arthritis (separately 11.8%) (Table 1).

Table 1. Comparison of complications in patients with acute or relapsed brucellosis.

Complications	Acute brucellosis N (%)	Relapsed brucellosis N (%)
Bilateral sacroilleitis	10 (25.6)	7 (29.1)
Unilateral sacroilleitis	3 (7.6)	4 (16.6)
Spondylitis	2 (5.1)	6 (25)
Spondylodiscitis	7 (17.9)	4 (16)
Paraspinal abscess	2 (5.1)	0
Arthritis	7 (17.9)	0
Neurobrucellosis	2 (5.1)	0
Abortion	1 (2.5)	0
Central vein thrombosis	1 (2.5)	0
Hepatitis	0	1 (4.1)
Epdydimo-orchitis	4 (10.2)	2 (8.3)

The historyof animal contact in acute and relapsed brucellosis was meaningfully higher than control group ($P = 0.030$); however, non- pasteurized dairy products consumption had no meaningful difference among groups ($P = 0.344$).

The mean serum level of vitamin D was higher in relapsed brucellosis (82.26) than control group (24.44), and in control group was higher than acute brucellosis (22.5), but on the basis of unilateral variance analysis there was no meaningful difference among three groups (Table 2).

The mean serum levels of 25-OH- VitD3 by male gender in acute brucellosis, relapsed brucellosis and healthy control groups were 20.35 ± 13.10, 24.88 ± 20.89 and 22.52 ± 13.79, respectively. Although, there was no statistically significant difference among three groups ($P = 0.442$) (Table 3).

There was no statistically significant difference between the mean serum level of 25-OH- VitD3 in patients with acute or relapsed brucellosis 24.69ng/mL with healthy persons 24.44 ng/mL ($P = 0.913$) (Table 4). There was no meaningful difference between acute brucellosis, relapsed brucellosis and healthy persons *(P* = 0.937) (Table 5). The mean serum levels of 25-OH-VitD3 in complicated and uncomplicated brucellosis were 25.01 ± 23.79 and 24.54 ± 16.12 ng/mL, respectively. However, there was no statistically meaningful difference between two groups ($P = 0.866$).

Discussion

Brucellosis is a bacterial zoonosis [2,3]. Iran is an endemic area and the incidence rate of brucellosis in Hamadan province, west of Iran is high; for example,in 2014, the incidence rate of brucellosis was 91 per 100,000 population, which approximatelywere threefold of the incidence rate in Iran (21 per 100,000) in the same year [3,5].

In brucellosis, due to intracellular microorganism, phagocytic activity of polymorphonuclear and

Table 2. Comparison of serum levels of 25-OH- VitD3 in acute brucellosis, relapsed brucellosis, and healthy persons.

Study groups	Serum level of 25-OH- VitD3 (ng/mL)				*P* value
	Mean	Standard deviation	Minimum	Maximum	
Acute brucellosis	22.55	15.72	< 10	81.80	
Relapsed brucellosis	26.82	20.78	< 10	90	0.275
Healthy individuals	24.44	17.29	< 10	102	

Table 3. Comparison of serum levels of 25-OH- VitD3in three groups by gender.

Gender	Control		Acute brucellosis		Relapsed brucellosis		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Female	28.41	22.58	25.29	15.72	30.45	20.78	0.447
Male	22.52	13.79	20.35	13.10	24.88	20.89	0.442

SD: Standard deviation.

Table 4. Comparison of serum levels of 25-OH- VitD3 in patients with brucellosis (acute and relapsed) and healthy persons.

Study group	Serum level of 25-OH- VitD3 (ng/mL)				*P* value
	Mean	Standard deviation	Minimum	Maximum	
Brucellosis (acute and relapsed)	24.69	18.50	< 10	90	0.913
Healthy individuals	24.44	17.29	< 10	102	

Table 5. Comparison of serum levels of 25-OH- VitD3 in three groups.

Study groups	Serum level of 25-OH- VitD3 (ng/mL)			*P* value
	Deficient < 20	Insufficient 20-29	Sufficient ≥30	
Acute brucellosis	48 (52.2%)	22 (23.9%)	22 (23.9%)	
Relapsed brucellosis	44 (47.8%)	24 (26.1%)	24 (26.1%)	0.937
Healthy individuals	54 (50.9%)	29 (27.4%)	23 (21.7%)	

momonuclear cells including macrophageshas main role in disease improvement [1].

Immune cells have 25-OH- VitD3 receptor, and a patient with vitamin D deficiency has disturbances in immune system,specifically macrophages. Twenty five-hydroxyvitamin D3 (25-OH- VitD3) stimulates osteogenic activity and regulates calcium metabolism, and also involves in host defense against microbial or inflammatory invasions [8,9].

In the current study, the mean serum levels of vitamin D3 were compared in three matched groups (based on gender, age and habitat). This study was done on the basis of this theory and relation of vitamin D and immune system. According to our findings, the mean value of serum level of vitamin D in acute brucellosis was lower than control group, and in the control group was lower than relapsed brucellosis. In addition, serum level of 25-OH- VitD3 in complicated brucellosis was higher than uncomplicated brucellosis, but there was no meaningful difference among acute brucellosis, relapsed brucellosis and healthy persons as well as between complicated and uncomplicated brucellosis. The only study about effect of serum level of vitamin D on brucellosis was done by Kurtaran*et al*.[16] between 2009 to 2010 in Turkey; they have reported that the serum level of 25-OH- VitD3 in brucellosis patients was meaningfully lower than control group. Researchers concluded that 25-OH- VitD3, probably has main role in pathogenesis of brucellosis. In addition,vitamin D3 level was lower in brucellosis patients compared to the control group and in males, vitamin D3 level was higher in the control group than in the patient group[16]. In our study,the mean serum levels of 25-OH- VitD3 in acute brucellosis, relapsed brucellosis and healthy control groups by gender (male and female) had not statistically significant difference; however, most of them had vitamin D3 insufficiency.That is maybe due to, low sun exposure in Iran because of the clothing type in women and men required under current law.Moreover, Hamadan province lies in an elevated region, with 'Alvand' mountains, running from the north west to the south west in Iran and it has warm summer and cold winter.

About the relationship between vitamin D deficiency with other infectious Diseases such as mycobacterium tuberculosis, an intracellular microorganism, multiple studies have been done, that showed a relationship [17-19].

In other study by Talat *et al*. in 2010 in Pakistan, on relationship between vitamin D deficiency and progression of tuberculosis; most of patients were vitamin D deficient [17].

In Izadpanah *et al.* study 2013 in Iran, patients with low serum level vitamin D, had prolonger hospital admission, higher nosocomial infections, including blood stream and respiratory infections [18]. Vitamin D3 induces the innate immune system and modulates B and T lymphocytes functions, and also influences inflammatory responses in inflammatory diseases involves in the host defense against microbial invasions [8-10].

As Nseir *et al*. reported in 2013, the relationship between serum level of vitamin D3 and recurrent urinary tract infections in premenopausal women, the mean serum level of 25-OH- VitD3 in women with recurrent UTI was meaningfully lower than control group [19].

In another study by Aregbesola *et al*. in 2013 in Finland, low serum level of 25-OH- VitD3 was a risk factor for pneumonia [20].

The immune system cells have 25-OH- VitD3 receptor and the effect of vitamin D deficiency on immune system is known; therefore, it seems the role of vitamin D deficiency in some diseases such as tuberculosis, pneumonia, female urinary tract infection, and nosocomial infections is provable [17-20]. In addition, vitamin D deficiency is associated with autoimmune diseseas such as multiple sclerosis and diabetes mellitus type 1[21,22]. In the study by Djukic *et al*. in 2014, showed that mortality and neurologic complications were high in E. coli meningitis and meningoencephalitis patients with vitamin D hypovitaminoses [23]. The results of our study have not accordance with the above studies.

However, in the present study, the serum level of vitamin D in relapsed brucellosis was slightly higher than healthy persons. Consumption of dairy products is common transmission rote of brucellosis in Iran [24,25]. According to the relation between serum level of 25-OH- VitD3 and dairy products consumption [8,26]; it seems that consumption of non-pasteurized dairy products had mutual influence on pathogenesis of brucellosis and elevation of serum level of 25-OH-VitD3 in the patients.

Vitamin D deficiency is common in Iran because of unhealthy lifestyle habits such as low sun exposure and physical activity and maybe low dairy consumption [27-29]. In addition, in Iran, exposure to sun is limited due to the type of clothing in women and men required under current law. Hence, it is predictable that the differences in serum level of 25-OH- VitD3 in the three groups, were not meaningful and our study is contrary to Kurtaran *et al* .study inTurkey [16].

Conclusion

According to the results, the prevalence of vitamin D deficiency was high in the patients with acute or relapsed brucellosis and also healthy persons; however, there was no meaningful difference among three groups. In contrary of previous study of Kurtaran *et al*., our findings showed that vitamin D deficiency is not probably a predisposing factor for acquisition or relapseof brucellosis. We purpose to investigate further studies on this subject and also vitamin D receptor level.

Acknowledgements

The authors would like to thank Vice Chancellor for Research and Technology Hamadan University of Medical Sciences for their financial support. Also, we would like to acknowledge the staff of Arad laboratory for their cooperation. This article was extracted from the approved thesis in Ethics Committee of Hamadan University of Medical Sciences by numbering ethical code IR.UMSHA.REC.1394.556.

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Conflict of interests: No conflict of interests is declared.