Core stability exercises on pain, lumbar and abdominal resistance in patients with brucellosis. Pilot study

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Summary

Introduction: Brucellosis is a zoonotic intracellular bacterial infection, whose most frequent complications are low back and abdominal pain. In addition, antibiotic therapy against *Brucella* has side effects that can exacerbate muscular pain in the lumbar area causing a deterioration of mobility and function of the lower extremities. For all these reasons, non-pharmacological interventions through therapeutic exercise are necessary to mitigate them. Our study has evaluated the effects of exercise on pain and resistance of the lumbar and abdominal musculature in patients with brucellosis.
Material and method: A prospective, randomized, single-blind clinical trial followed the Consolidated Standards of Reporting Trials (CONSORT) norms. After the screening, 24 patients with brucellosis were randomized to a core stabilization exercise training (CSE) group (n = 15) of the multifidus and transverse abdominal muscles (2 weeks / 3 sessions /10 minutes/day) and a control group (CON) (n = 9) with no training. Pre- and post-CSE testing included endurance of the lumbar and abdominal musculature, using the modified Biering-Sørensen test and abdominal crunch tests. In addition, the visual analog scale (VAS) was used to measure perceived pain in brucellosis patients.
Results: The resistance of the abdominal and lumbar musculature increased significantly ($P = 0.001$) in the CSE group after 2 weeks of training at the baseline and to the CON. In addition, VAS decreased significantly ($P = 0.001$) in the CSE group with respect to the CON.
Conclusion: Our results could indicate that a CSE training program significantly improves the resistance of the lumbar and abdominal musculature and decreases the perception of pain in patients with brucellosis. Therefore, it would be appropriate to recommend CSE protocols during recovery from *Brucella* infection.

Key words:
Brucella. Low back pain. Core stabilization exercise.

Ejercicios del *core* sobre dolor, resistencia lumbar y abdominal en pacientes con brucelosis. Estudio piloto

Resumen

Introducción: La brucelosis es una zoonosis bacteriana intracelular, cuyas complicaciones más frecuentes son el dolor lumbar y abdominal. Además, la terapia antibiótica contra la *Brucella* puede exacerbarlo provocando un deterioro de la movilidad y la función en las extremidades inferiores. En consecuencia, es necesario intervenciones no farmacológicas mediante ejercicios de estabilización de la zona central del cuerpo (*core*) (CSE) que implican el fortalecimiento de los músculos profundos lumbares y abdomen, que proporcionan soporte y estabilidad a la columna vertebral. Nuestro estudio ha evaluado los efectos de CSE sobre el dolor y la resistencia del *core* en pacientes con brucelosis.
Material y método: Se ha realizado un ensayo clínico prospectivo, aleatorizado y simple ciego siguiendo las normas *Consolidated Standards of Reporting Trials* (CONSORT). Tras un cribado, 24 pacientes con brucelosis se distribuyeron aleatoriamente a un grupo de entrenamiento de CSE (n = 15) de los músculos multífidos y transverso abdominal (2 semanas / 3 sesiones /10 minutos/día) y un grupo control (CON) (n = 9) sin entrenamiento. Las pruebas previas y posteriores al CSE incluyeron resistencia de la musculatura lumbar y abdominal, mediante las pruebas modificada de *Biering-Sørensen* y de abdominales. Además, se utilizó la escala visual analógica (VAS) para medir el dolor percibido en los pacientes con brucelosis.
Resultados: La resistencia de la musculatura abdominal y lumbar aumentó significativamente ($p = 0{,}001$) en el grupo CSE tras 2 semanas de entrenamiento con respecto al inicio del estudio y también frente al CON. Además, VAS disminuyó significativamente ($p = 0{,}001$) en el grupo CSE con respecto al CON.
Conclusión: Nuestros resultados podrían indicar que un programa de CSE mejora significativamente la resistencia de la musculatura lumbar y abdominal y disminuye la percepción de dolor en pacientes con brucelosis. Por tanto, sería es adecuado recomendar protocolos de CSE durante la recuperación de la infección por *Brucella*.

Palabras clave:
Brucella. Dolor lumbar. Ejercicios de estabilización del *core*.

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Introduction

Brucellosis is a zoonotic intracellular bacterial infection caused by nonmotile aerobic Gram-negative coccobacillus of the genus *Brucella*. Three classic species (*B. mellitensis, B. abortus,* and *B. suis*) responsible for human brucellosis are recognized, with specificity of animal species, geographical distribution, and pathogenic peculiarities[1]. Humans generally acquire the disease through direct contact with infected animals, by eating or drinking contaminated animal products such as unpasteurized dairy products, or by inhaling airborne agents[2]. Brucellosis infection can affect any body part, including the reproductive system, liver, heart, and central nervous system[3]. Symptoms of Brucellosis typically include typhoid fever, muscle pain and fever, swelling of the knee or pelvis in children, or chronic fever with back and pelvic pain in adults[3]. Approximately 50% of patients with Brucellosis present episodes of lumbar or pelvic-abdominal pain[4]. Low back pain (LBP) has been considered a predictor of local impairment, among other factors[5]. Kuruoglu, *et al.*[6] recently reported that the risk of clinical progression and complications could be predicted by the presence of symptoms such as myalgia or low back pain in *Brucella*-infected patients.

Brucellosis treatment usually lasts several months, requiring 2 antibiotics (doxycycline or trimethoprim/sulfamethoxazole (TMP/SMX) plus gentamicin, streptomycin, or rifampicin)[7]. Antibiotic treatment has serious side effects, including those resulting in death, prolonged hospitalization, discontinuation of therapy, or persistent or significant long-term disability, and minor adverse reactions, such as gastrointestinal upset, central nervous system events, hepatotoxicity, nephrotoxicity, ototoxicity or dermatitis[7]. These side effects may exacerbate complications related to local episodes of joint, muscle, and LBP, potentially leading to impaired mobility and function of the lower extremities, particularly in women[8].

Thus, patients with brucellosis need to enhance muscular endurance and strength more than healthy people because *Brucella* disease implies a progressive and symmetrical muscle loss of strength for both extremities, with subsequent inability to move[9]. Consequently, public health professionals are making great efforts to combat physical inactivity-related diseases, although without yet significantly increasing physical activity levels and behavioral changes in these patients[10].

Therefore, planning an effective sports medicine rehabilitation involves addressing in a comprehensive manner exercises beneficial for LBP, through core stability training to increase strength and subsequently muscular and spine endurance[11]. These exercises are defined as the ability to control the position of the trunk on the pelvis for efficient production and transfer of force to the distal parts of the body during exercise[12]. The muscles in this anatomic area include some of the trunk and thigh muscles responsible for maintaining the stability of the spine and pelvis, which are crucial for transferring energy from the trunk to the upper and lower extremities[13] core muscle stability is a crucial component of any large-scale motor activity[14], the importance of which has also been recognized by researchers in the etiology and treatment of musculoskeletal injuries[15]. Due to the nature of brucellosis, the seemingly optimal approach is to identify and design programs that reduce the acute symptoms of the disease and improve motor abilities[16]. However, more than one symptom in patients with *Brucella* must be considered, which requires a thorough evaluation and a specific sports medicine rehabilitation program that involves benefits on the spine[17]. In this way, core stabilization protocols are ideal for balance improvement[18], individuals with problems in the lower extremity[19], inactive elderly individuals[20], women with multiple sclerosis[21], LBP alleviation[22], and sports injury prevention[23]. Thus, core exercises strengthen the musculature provide stability to the spine and show promising outcomes[15]. In this sense, these findings could relate specifically to brucellosis patients, as core stabilization exercises (CSE) could reduce the amount of pain and muscle weakness during hospitalization and decrease the use of drugs and treatment time.

To our knowledge, no therapeutic physical exercise programs, using sports medicine rehabilitation criteria, in patients with *Brucella* can help alleviate the fatigue, acute pain, and muscle weakness that most patients experience[22]. For all the above, the use of core stability training protocols can be effective in strengthening the core muscles and reducing pain in patients with brucellosis. Our research aimed to find out whether the application of a CSE-based rehabilitative sports medicine treatment approach on the lumbar and abdominal muscles in patients with LBP by *Brucella* infection, conducted at Valiars Hospital in Zanjan (Iran), results in an improvement in physical parameters.

Material and method

Study design

A single-center randomized clinical trial was conducted at Valiars Hospital in Zajan (Iran). The study was reported according to CONSORT (Consolidated Standards of Reporting Trials) updated guidelines for reporting parallel group randomized trials[24] and approved by the Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1398.093). This project was prospectively registered at https://www.irct.ir/trial/38104 (IRCT20190225042842N1) and all subjects provided written informed consent after participants were presented with full explanations of the study's objectives and treatment protocol, following the Declaration of Helsinki and the 2013 Fortaleza revision[25].

Patients

Twenty-four participants aged between 36 and 40 years old with brucellosis were enrolled in this study. The reason for choosing the age range of the patients was chosen based on the statistics of those who referred to this medical center. Furthermore, *Brucella* can affect anyone regardless of age and the patient's age does not seem to be related to the consequences of the pathology. Participants were randomized and assigned to 2 groups: the core stabilization exercise (CSE) group, and the control (CON) group, according to the date of entry into the study using a system of balls that were placed in a lottery container and then randomly removed from the container without replacement to record the resulting sequence. The assignment was performed by an independent researcher who was not involved in other experimental procedures.

The sample size was selected by reference to previous studies[20–23] and the available patients, given the preliminary and innovative nature of the plan based on the use of rehabilitation sports medicine exercises.

The participants were volunteers from patients admitted to the infectious diseases department of Valiasr Hospital in Zanjan (Valiasr Hospital, Zanjan, Iran) whose disease was diagnosed in the first six months of 2019, according to the guidelines for combating brucellosis[26]. It is worth noting that all hospital measures concerning hospitalization conditions for the prevention of COVID-19 were observed and in case of severe lumbar pain, simple oral analgesics such as acetaminophen were administered. All patients started on the same antibiotic treatment regimen and used the same analgesics when required. Antibiotic and analgesic therapy was prescribed and supervised by the responsible physician.

Inclusion / exclusion criteria

The participants were selected according to the following inclusion criteria: adults (males and females) aged 18-71 (age range of patients who attended Valiars Hospital in Zanjan (Iran), presenting clinical symptoms consistent with brucellosis, a Wright test titer greater than 1:160, a 2ME test (2-mercaptoethanol *Brucella* agglutination test) titer greater than 1:40, acute LBP, lack of professional physical activity during the past 2 years, no other infectious diseases, and proper diet. The exclusion criteria comprised a history of pelvic and spinal cord surgery, cancer, history of fractures and discopathy in the spine, known renal, hepatic, pulmonary, and/or cardiac diseases, severe neurological diseases, and consumption of alcohol and/or narcotics and/or psychotropic drugs.

Core stabilization exercises protocol

Core stabilization exercises protocol was designed based on the principles of sports medicine rehabilitation techniques. Exercises were performed for 10 minutes (min) in the morning, at noon, and at night. All movements had to be performed ten times in a painless area. The exercises were performed as follows[27]:

After collecting pre-test data, the experimental group underwent core muscle stabilization training for 2 weeks while hospitalized. These exercises were performed under the supervision of a sports medicine expert and a physical education expert in 3 sessions of 10 min per day. A warm-up phase (consisting of light power stretching) was performed at the beginning of each treatment session. The principle of overload in each exercise was considered and determined concerning the correct performance and pressure of the exercise in the previous session. The main emphasis was on the multifidus and transverse abdominal muscles, essential for proper load balance within the spine, pelvis, and kinetic chain. The study procedures are represented in Table 1.

Outcome measures

Physical parameters

In the pre-test and post-test, the Sørensen test and sit-up tests were used, respectively, to measure lumbar and abdominal muscle endurance. To evaluate the stiffness of the back muscles, the modified Biering-Sørensen test of Static Muscular Endurance (BSME)[28] was used.

The time that the subject could maintain the horizontal position was recorded. When the horizontal position of the body was disturbed or the subject grabbed the bench with his hands, the stopwatch time was stopped and the duration of maintaining the position was recorded as the endurance score of the spinal extensor muscles in the information registration form. The pre-test and post-tests were recorded. The internal reliability of this test was reported as an intraclass correlation coefficient (ICC) = 0.97 for healthy individuals and ICC = 0.97 for patients suffering from back pain[29]. The patients were hospitalized under medical control and trained under the supervision of a sports medicine physician.

Perceptual pain

The Visual Analogue Scale (VAS) questionnaire[30] was used to assess participants' pain perception. This scale measures pain intensity using a poster the length of a 50 cm ruler with the word "painless" at the left end and "the most severe pain" at the right end. Over the past 48 h, the individuals estimated their pain levels on a continuum scaled from 0 to 10. In a previous study[31], Cronbach's alpha coefficient, the sum of reliability of test-retest scores, and the inter-rater reliability were 0.91, 0.89, and 0.90, respectively, for this questionnaire.

Table 1. The treatment protocol was performed by the core stabilization exercise (CSE) group and control group (CON).

Groups	Procedures
CON	**Routine antibiotic treatment** Rifampicin, doxycycline, tetracycline, gentamicin, trimethoprim/sulfamethoxazole
CSE	**Same antibiotic protocol as the control group plus CSE** • In the recumbent position, close each leg to the abdomen with the hands and maintain the stretch for six seconds. • In a recumbent position with a hands-on chest, the legs should shuttle in tandem with each other 10 times. • With the help of hands and feet, lift the waist and hips and continue for 6 s. • Horse position: Maintain lumbar contraction for six seconds. • Cat position: Maintain abdominal contraction for six seconds. • In the prone position with elbows resting on the ground, lift the head and chest and hold for six seconds. • Four-legged position. Contract the abdomen and attach the waist to the ground. Hold this position for 6 s.

CSE: Core stabilization exercise; CON: Control group.

Statistical analysis

All data in the figures and table represent the mean and standard deviation. The Shapiro-Wilk test was used to determine the normality of data. Descriptive statistics, and independent sample t-test, were used to analyze participant characteristics. Within group comparisons of all data were analyzed using the paired t-test. Between-group comparisons were calculated using analysis of covariance (ANCOVA) for adjusting the baseline data. Data analysis was performed using SPSS version 20 software (IBM Corporation, Armonk, NY, USA) at a significance level of $P \leq 0.05$.

Results

Sample and characteristics

A total of 84 participants were assessed for eligibility by the infectious diseases department of Valiasr Hospital in Zanjan. 38 patients were excluded from participating; 24 of these were unwilling to participate and another 14 did not meet the other inclusion criteria. Eight patients in the CSE group and 14 patients in the CON group dropped out. The remaining 34 subjects completed the study without incident (Figure 1).

The demographic data of this study are shown in Table 2. There was not statistically significant ($P < 0.05$) difference in age (years), Height (Cm), Weight (Kg), BMI (Kg/m^2), perceived pain (score), and abdominal and lumbar endurance among the groups.

Pain perception

The participants' pain perception significantly differed between the groups in the post-test ($F = 7.58$, $P = 0.001$, PES= 0.369). Compared with the pre-test phase, pain perception decreased significantly in the CSE group than CON group after CSE. The mean scores of the pre-test and post-test pain perception did not considerably change in the CON group (Figure 2, panel A).

Abdominal and lumbar muscle endurance

The CSE group significantly increased abdominal muscle endurance ($F = 6.36$, $P = 0.001$, PES = 0.302). The CSE group led to a significant increase in abdominal muscle endurance compared to the pre-test. In contrast, abdominal muscle endurance remained almost constant at the two stages in the CON group and did not change significantly (Figure 2, panel B).

Lumbar muscle endurance significantly increases in the CSE group compared to the CON group ($F = 7.66$, $P = 0.001$, PES = 0.158). There was no significant difference between the mean pre-test and post-test in the CON group (Figure 2, panel C).

Figure 1. CONSORT[24] flowchart of participant recruitment and disposition throughout the study.

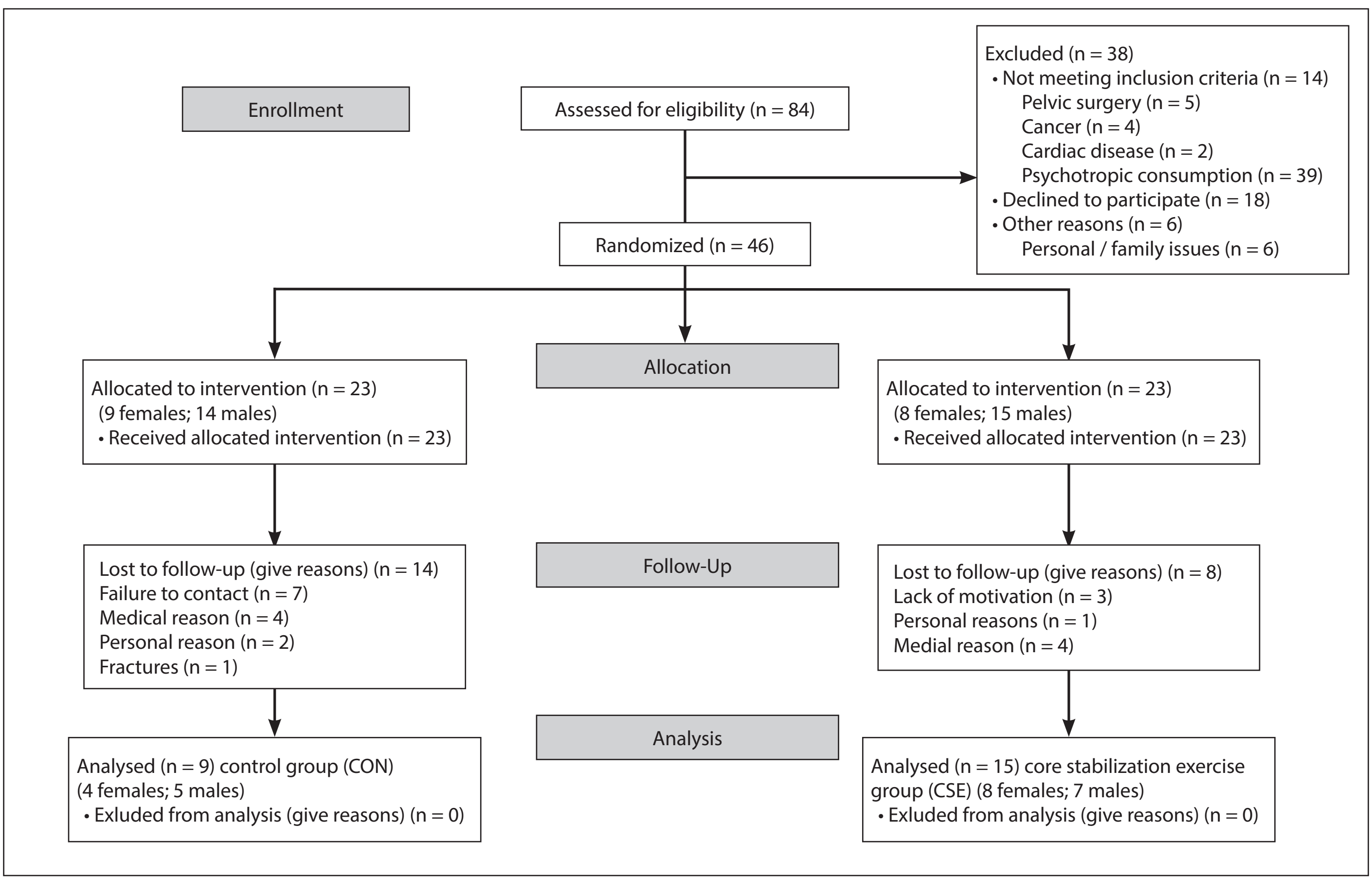


Table 2. Characteristics of enrolled participants.

Characteristics	CSE group (n = 8 females, 7 males)	CON group (n = 4 females, 5 males)
Age (year)	38.11 ± 1.78	37.78 ± 1.38
Height (Cm)	164.65 ± 4.86	162.28 ± 3.44
Weight (Kg)	69.57 ± 2.93	71.32 ± 2.71
BMI (Kg/m^2)	27.78 ± 1.78	26.39 ± 1.89
Perceived pain (score)	8.05 ± 1.44	7.96 ± 1.57
Abdominal endurance (number per minute)	1.78 ± 0.02	1.96 ± 0.13
Lumbar endurance (minutes)	3.56 ± 0.44	3.10 ± 0.51

BMI: Body mass index (calculated as weight in kilograms divided by height in meters squared); CSE: Core stabilization exercises; CON: Control. Data are expressed as the mean ± standard deviation. Note: There were no significant differences (P >0.05) between groups.

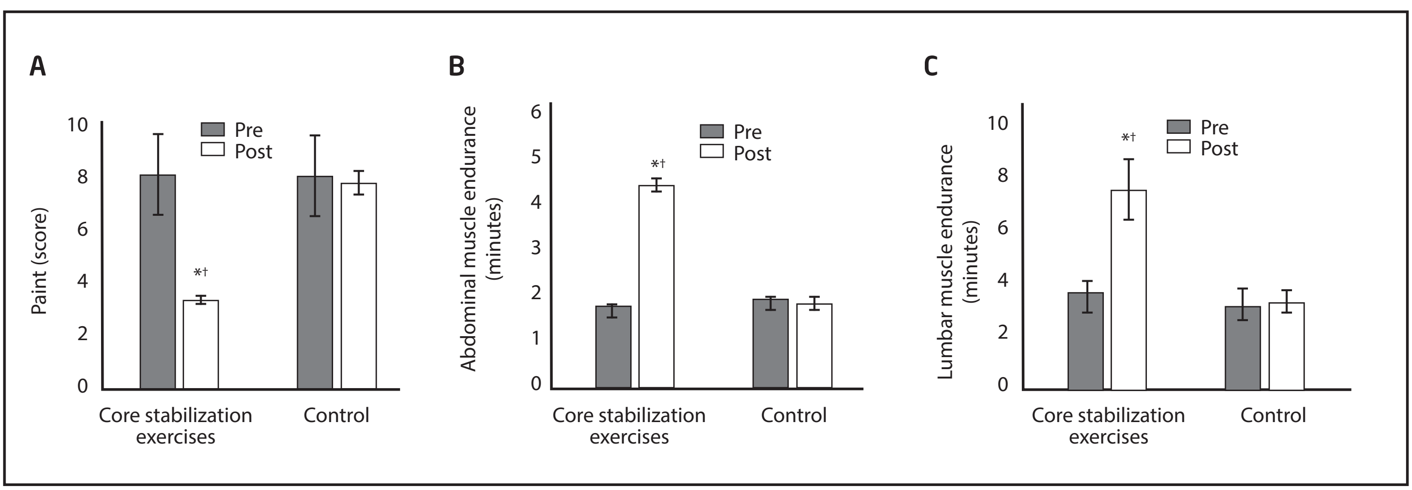
Discussion

The present study aimed to determine the effect of lumbar and abdominal CSE on the pain and endurance of the lumbar and abdominal muscles in patients with brucellosis and acute LBP. The results of the covariance test showed that a period of lumbar core stabilization training had a significantly positive effect on pain reduction and abdominal and lumbar muscle endurance enhancement. These results align with other studies[32–34] that showed that trunk stability exercises effectively increase trunk muscle strength and endurance, reducing pain with superior results to strengthening exercises.

Two weeks were considered for patient treatment and direct access to achieve adequate follow-up after initial treatment and reduction of pain symptoms. This study used exercise as an adjuvant therapeutic aid, and patients have also received clinical treatments at the same time, as in other diseases caused by infections[35]. In addition, this method was implemented as a pilot study for the first time and was supposed to be followed by more accurate studies in the future if minimal effects were observed throughout clinical and pharmacological interventions. However, probably not all pain reduction is due to exercise. Some initial neuromuscular adaptations, such as the coordination of muscle spindles and Golgi tendon organs, can occur through the initial stimulation of the two-week training[36].

Brucellosis can affect almost all organs in the body, and hence complications may develop. Some patients have chronic brucellosis and have symptoms for years, even after treatment, which can lead to skeletal and joint disorders[21]. One of the causes of pelvic and lumbar pain in these patients is the disease itself (*Brucella*), which releases inflammatory agents in the body[3]. The inflammatory response produced by brucellosis will result in the release of platelet-activating factor (PAF), increased expression of phospholipase A2, and rapid formation of reactive oxygen species (ROS). The phosphatidylcholine (PC) of the *Brucella* envelope, having a cyclic fatty acid at C2, is susceptible to hydrolysis by phospholipase A2 and oxidation by ROS, amplifying the inflammatory response, leading to the release of inflammatory mediators such as interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, and macrophage chemoattractant protein-1[37,38]. In addition, inactivity, lack of exercise, and muscular weakness caused by this disease can exacerbate pelvic and lumbar pain in patients[39]. Complications of the skeletal muscular system include arthritis, spondylitis, osteomyelitis, tenosynovitis, and bursitis, which commonly involve the hip, knee, wrist, shoulder, and lumbosacral joints[40]. The lumbosacral vertebrae are involved in most patients with articular inflammation, leading to lumbar pain that spreads to the feet. In such conditions, immunologic inflammation of the joint spreads to the large and small joints and increases pain. In addition, brucellosis can invade the intra-vertebral disc and lead to adjacent osteomyelitis of the two vertebrae; the lumbar pain of these conditions may extend down to the tibia, resulting in impaired walking[41].

Figure 2. Results of pain, abdominal and lumbar muscle endurance.



The mean difference changes in the perceived pain (panel A), abdominal muscle endurance (Panel B), and lumbar muscle endurance (panel C) after 2 weeks of core muscle stabilization (training and controlled conditions). Analysis of covariance (ANCOVA) for adjusting the baseline data. Data are expressed as the mean ± standard deviation. Abbreviations: Pre: start of study; Post: end of study. Symbols: *: significant difference between groups; †: significant difference between Pre and Post.

As shown by the results of the CSE group, patient pain perception levels were significantly reduced after implementing the designed protocols. Simultaneously, the endurance of their abdominal and lumbar muscles increased significantly compared to the pre-test and the control group. From these results, it can be inferred that the function of the core muscles influences the level of pain and endurance of the lumbar–abdominal muscles. According to Zahedpour, *et al.*[42] core stability training decreased the mean intensity of low back pain in postpartum women (as measured by VAS and Quebec Back Pain Questionnaire) by 72.8% and 74.2%, respectively, whereas the control group reported a 1.8% and 1.6% increase in pain, respectively. These results are in line with the evaluation of the effects of health education interventions on promoting knowledge, beliefs, and preventive behaviors in patients with brucellosis. They have shown that training people based on the health belief model (HBM) constructs significantly affects people's knowledge, attitudes, and practices with brucellosis and cancer[43,44], suggesting interventions and programs for people with brucellosis, which have strong theoretical foundations. Perceived self-efficacy is the most crucial construct affecting the health belief model for brucellosis. A significant difference between perceived self-efficacy and the performance of brucellosis patients before and after educational interventions were reported[45].

Prevention of brucellosis may not be possible, but management and control of disease-related complications such as perceived pain and muscle performance can be modified by CSE. Therefore, these findings can be generalized to the efficiency of CSE in the management of brucellosis complications[46]. Therefore, decreased pain perception and improved muscle performance can be generalized to CSE in brucellosis patients. Fatigue in large muscle groups can reduce muscle coordination and function while also affecting postural function and control in individuals. Santos, *et al.*[47] investigated the effect of a core muscle fatigue protocol on the pedaling mechanics of professional cyclists, concluding that core muscular fatigue caused changes in the pedaling mechanics and alignment of the lower extremities. The concept of fatigue has been discussed since the feeling of pain, weakness, and fatigue itself in muscles can be a symptom of brucellosis[48]. Researchers have suggested that increased core muscle stability and endurance could improve lower extremity alignment during daily activities and exercise[47].

Therefore, exercises that enhance endurance and improve their ability to cope with fatigue can be recommended considering the wear and tear possibilities of the core muscles during brucellosis. This method may prevent reductions in extremity function during the disease because decreased lumbar-abdominal muscle endurance and strength are common causes of pain for these patients. Hart, *et al.*[49] observed decreased quadriceps muscle activity following fatigue in the trunk extension muscles in healthy subjects and patients with LBP[49]. Subsequently, Distefano, *et al.*[50], in a review of the effect of core stability exercises on healthy youth balance, reported that core stability exercises were influential in balance performance improvement. The experimental groups performed better after core stabilization exercises than the control group[50]. According to core body strengthening and limb movement theory[51], fatigue in the body's core muscles can lead to reduced motor functions. Therefore, CSE can help reduce the pain and endurance of the lumbar and abdominal muscles associated with spinal and pelvic control.

The results of this study suggest that increased core endurance training should be used in treatment plans for patients with brucellosis to prevent reduced extremity function due to the progressive and symmetrical loss of muscle strength[52]. However, it is worth noting that factors such as depression, anxiety, and stress also affect patient attitudes and physical activity levels[53].

Patients with brucellosis may find it difficult to change their physical activity patterns at the request of others. Therefore, it is crucial to develop effective strategies to increase activity in patient populations. Health-related professionals can often enhance physical mobility in daily patient routines through dual strategies of changing the physical environment around them and providing free quality services. Increasing the health-promoting behaviors of patients with brucellosis is necessary to reduce their health and social costs[54]. The ways and means of effectively promoting physical activity in brucellosis patients still present essential questions, necessitating future research to address these challenges and alter patient physical and behavioral processes[55].

Limitations

This study has some limitations. The protocol was designed to minimize sources of bias; however, given the nature of the intervention, it was not possible to blind the treating therapists and patients. Another limitation is the small number of participants, using the patients available in the Hospital without calculation of the sample size. Likewise, the short intervention period, and the lack of follow-up of the patients after hospital discharge were other limitations of the present study. Furthermore, the cause of low back pain can also be multifactorial and biopsychosocial. For these reasons, it is recommended to interpret the results with caution. Therefore, to accurately understand the effect of CSE, it is suggested to examine the effect of CSE in a larger population of brucellosis patients over a longer period, at least eight weeks with regular follow-up. These future studies could be carried out in non-hospitalized patients in chronic brucellosis periods and even after treatment.

Conclusion

The present study showed that CSE could reduce pain and improve lumbar and abdominal muscle endurance in a patient's brucellosis. Therefore, CSE is recommended alongside identifying other medical orders to increase the quality of life of the brucellosis patient. The value of aggressive surgical and/or antimicrobial therapy in conjunction with a comprehensive rehabilitation program during hospitalization is emphasized in these patients to provide optimal pain management and prevent further disability. Overall, there are few published reports on the exercise outcomes of the brucellosis patient.

Conflicts of interest

All authors declared that there are no conflicts of interest.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS. REC.1398.093). This project was prospectively registered at https://www. irct.ir/trial/38104 (IRCT20190225042842N1).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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