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Research Article

Increased DNA Damage and Oxidative Stress in Patients with Brucellosis

Hasan Karsen[1]*, İsmail Koyuncu[2], İrfan Binici[1], Emine Ayca Güler[1], Hakim Çelik[3] and Şahbettin Selek[4]

[1]*Department of Infectious Diseases, Faculty of Medicine, Harran University, Şanlıurfa, Turkey*

[2]*Department of Biochemistry, Faculty of Medicine, Harran University, Şanlıurfa, Turkey*

[3]*Department of Physiology, Faculty of Medicine, Harran University, Şanlıurfa, Turkey*

[4]*Department of Biochemistry, Bezmialem Vakıf University, İstanbul, Turkey*

***Corresponding Author:** Hasan Karsen, Department of Infectious Diseases, Faculty of Medicine, Harran University, Şanlıurfa, Turkey.

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Abstract

Background: Our aim is to investigate whether brucellosis causes carcinogenesis through studying the oxidative/anti-oxidative level and DNA damage in different serological titers of brucellosis.

Material and Methods: In the different serological test titers of brucellosis standard agglutination test (SAT): 1/40 = 12 people, 1/80 = 13 people, 1/160 = 13 people, 1/320 = 12 people, 1/640 = 12 people) and 19 healthy people were included in the study. DNA damage was investigated through 8-hydroxy-2'-deoxyguanosine Cayman's ELISA kit method. The total oxidative status (TOS), total antioxidant capacity (TAC), total thiol (SH) and lipid hydroperoxide (LOOH) levels were analyzed through colorimetric method. Also, oxidative stress index (OSI) was found via calculation.

Results: It was realized that the TOS, LOOH, OSI and DNA damage gradually increased beginning from the control group and brucella SAT 1/40 titer and gained statistical significance (respectively at the 1/160, 1/640, 1/160, 1/80 titers). ($p < 0.001$). The TAC and SH however, was found to gradually decrease beginning from the control group and brucella SAT 1/40 titer and gain statistical significance (respectively, at the 1/320, 1/640 titers). ($p < 0.001$). There was a significantly positive correlation between serum brucella SAT titers and DNA damage, TOS, OSI and LOOH (respectively, *rho* = 0.796, 0.810, 0.389, 0.717). And there was significantly negative correlation between brucella SAT titers and TAC and SH (respectively, *rho* = -0.540, -0.503).

Conclusion: The serological antibody titer increased in brucellosis, oxidative stress and DNA damage also increased in relation to it. And this DNA damage might lead to brucellosis-related mutation and carcinogenesis. Therefore, these patients must be treated urgently to counteract the DNA damage.

Keywords: Brucellosis; Oxidative Stress; DNA Damage; Carcinogenesis

Introduction

Several infectious agents and chronic inflammation have been recognized as important risk factors for carcinogenesis and malignancies to humans [1-3]. The International Agency for Research on Cancer (IARC) has estimated that approximately 18% (1.8 million) of cancer cases worldwide are attributable to infectious diseases throughout the year [4]. Various viruses, parasites and bacteria which cause carcinogenesis and malignancies have been reported [5-7].

Brucellosis is endemic in many countries throughout the world, including Turkey. It is a chronic infectious and granulomatous disease caused by the *brucellae* bacteri that invades the multipl organ. Morbidity and sequelae due to brucellosis are high. Some parameters related to the paraoxonase enzyme and oxidative stress has been studied in brucellosis [8-10]; nevertheless, as far as we have searched, no studies have been conducted to investigate its relationship with carcinogenesis so far.

In the present study, we examined the relationship of oxidative/anti-oxidative level and DNA damage within groups in the healthy control group and different serological titers of brucellosis. Our purpose was to investigate if brucellosis was a risk factor for carcinogenesis.

Materials and Methods

A total of 62 brucellosis cases who were serologically diagnosed from the serum of patients through the standard agglutination test (SAT) and hadn't received any treatment and a control group of 19 healthy cases were included in the study.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000 and approved by the local eth-

ics committee. All subjects were informed about the study, and written consent was obtained from each subject.

Exclusion criteria

The exclusion criteria included a history of alcohol abuse, habitual smoking, intravenous drug abuse, pregnancy, the use of antioxidant supplements, hypertension, diabetes mellitus, liver or renal disease, rheumatoid arthritis and pulmonary disease.

Measurement of lipid hydroperoxide (LOOH) levels

Serum LOOH levels were measured with the ferrous ion oxidation–xylenol orange assay [11]. The principle of the assay depends on the oxidation of ferrous ion to ferric ion through various oxidants, and the produced ferric ion is measured with xylenol orange. LOOH's are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels.

Measurement of thiols activities

Serum total thiol concentration or sulfhydryl groups (SH) were measured by the methods originally described by Ellman [12] and modified by Hu [13]. Here, thiols interact with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), forming a highly colored anion with maximum peak at 412 nm (e412 = 13 600 M^{-1} 1 cm^{-1}). In this study, this method was adapted to an automated biochemistry analyzer (Advia 1800; Siemens, Germany) [14].

Measurement of total antioxidant capacity (TAC)

Serum TAC levels were determined using a novel automated measurement method developed by Erel [15]. In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing ferrous ion solution and hydrogen peroxide solution. In the most recently developed assay by Erel [15], same reaction is used. In the assay, ferrous ion solution, which is present in the Reagent 1, is mixed with hydrogen peroxide, which is present in the Reagent 2. The sequential-produced radicals such as brown-colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, anti-oxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got perfect precision values, which are lower than 3%. The results are expressed in mmolTrolox Equiv.L^{-1}.

Measurement of total oxidant status (TOS)

Serum TOS levels were determined using a novel automated measurement method developed by Erel [16]. In this method, oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity which can be measured spectrophotometrically is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv. L^{-1}).

Determination of oxidative stress index (OSI)

The ratio of TOS to TAC represents the oxidative stress index (OSI), an indicator of the degree of oxidative stress. The OSI value is calculated according to the formula: OSI (arbitrary unit) = TOS (μmol H_2O_2Eq/L)/TAC (mmolTroloxEq/L) x100.

DNA damage (8-OHdG) measurument

Venous blood samples were collected from each subject. The blood samples were placed on 4 C for 10 minute and then centrifuged at 3500 rpm, 4°C for 15 minutes, and the separated serum was stored at −80°C until assayed. Quantification of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was done using OxiSelect™ Oxidative DNA Damage ELISA Kit (cell biolabs). Protocol was followed essentially as described by the manufacturer for the quantification of 8-OHdG (ng/ml).

Statistical analysis

IBM SPSS version 23.0 software (Chicago, IL, USA) was used for data analysis. Parameters were expressed as mean ± standard deviation. Kolmogorov–Smirnov test was used for a normal distribution before analysis. One-way ANOVA and Bonferroni post hoc adjustment test was used to compare Titer and Control group. Spearman correlation test was used to evaluate the relationship between serum titers and TAC, TOS, total thiol, OSI and DNA Damage. P-values < 0.05 were considered significant.

Results

The age, gender and body mass index (BMI) features of the brucellosis cases and control group were statistically matched. These demographic features are presented in table 1.

Parametre	Control group and titres of brucella standard agglutination test (SAT)						
	Kontrol(n = 19)	1/40 (n = 12)	1/80 (n = 13)	1/160 (n = 13)	1/320 (n = 12)	1/640 (n = 12)	
Man/Woman	9/10	6/6	7/6	6/7	5/7	6/6	NS
BMI kg/m^2 (Mean ± SD)	21.12 ± 3.24	22.02 ± 2.26	21.12 ± 3.2	22.18 ± 0.26	21.32 ± 3.26	22.11 ± 1.24	
Age (Ortalama)	38.03 ± 16.10	37.22 ± 14.32	38.13 ± 06.10	37.2 ± 04.30	37.23 ± 15.10	38.2 ± 14.33	

Table 1: Demographic features of the control group and brucellosis cases.

In this study, it was found that compared to the control group, the total oxidative status (TOS) which was one of the molecules taking part in the oxidation gradually increased beginning from the brucella SAT 1/40 titer and gained statistical significance beginning from 1/160 titer ($p < 0.001$). It was also found that compared to the control group, lipid hydroperoxide (LOOH) as another molecule taking part in the oxidation gradually increased beginning from the brucella SAT 1/40 titer and gained statistical significance at the 1/640 titer ($p < 0.05$).

Nevertheless, it was determined that compared to the control group, the total antioxidant capacity (TAC) as one of the anti-oxidant molecules gradually decreased beginning from the brucella SAT 1/40 titer and gained statistical significance beginning from 1/320 titer ($p < 0.001$). Likewise, it was noticed that as another anti-oxidant molecule, the total thiol (SH) groups compared to the control group gradually decreased beginning from the brucella SAT 1/40 titer and gained statistical significance at the 1/640 titer ($p < 0.001$) (Graphic I). Compared to the control group, OSI was also found to gradually decrease beginning from the brucella SAT 1/40 titer and gained statistical significance beginning from the 1/160 titer ($p < 0.001$) (Graphic II). Related to the oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) which occurred as a result of DNA damage was gradually increased compared to the control group and gained statistical significance beginning from the 1/80 titer ($p < 0.001$) (Graphic III).

In the study, a significantly positive correlation was found between serum brucella SAT and DNA damage, TOS, OSI and LOOH ($p < 0.001$ and respectively, rho = 0.796, 0.810, 0.389, 0.717). In contrast, a significantly negative correlation was found between serum brucella SAT and TAC and SH ($p < 0.001$ and respectively, rho = -0.540, -0.503) (Table 2).

Parameters	Control (n = 19)	1/40 (n = 12)	1/80 (n = 13)	1/160 (n = 13)	1/320 (n = 12)	1/640 (n = 12)	ANOVA P Values
TOS	9,8 ± 0,42	11,1 ± 1,2	11,6 ± 2,1	13,3 ± 2,5*	14,6 ± 2,8*	16,4 ± 1,4*	<0.001
TAC	0,69 ± 0,18	0,61 ± 0,15	0,58 ± 0,17	0,52 ± 0,15	0,48 ± 0,11*	0,39 ± 0,13*	<0.001
OSI	1,51 ± 0,47	1,89 ± 0,46	2,13 ± 0,66	2,61 ± 0,53*	3,09 ± 0,68*	4,64 ± 1,84*	<0.001
SH	0,48 ± 0,17	0,43 ± 0,06	0,39 ± 0,09	0,32 ± 0,10*	0,31 ± 0,09*	0,28 ± 0,13*	<0.001
LOOH	2,71 ± 0,91	2,81 ± 0,93	2,98 ± 0,95	3,09 ± 0,85	3,43 ± 0,79	3,96 ± 1,05*	= 0.011
DNA Damage	2,05 ± 0,78	3,46 ± 2,32	5,83 ± 2,11*	6,31 ± 3,76*	8,28 ± 3,23*	10,17 ± 4,67*	<0.001

Table 2: Comparison of the oxidative stress and DNA damage parameters of the control group and brucellosis cases

*P is significant.

Discussion

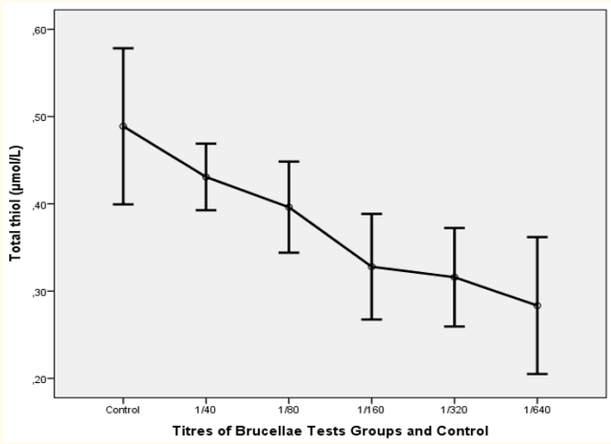
Previous studies highlighted that various viruses (HBV, HCV, HPV, EBV etc.), parasites (*Fasciola hepatica, S. haematobium* etc.) and bacteria *(H. pylori*) lead to malignancies [2]. Beside these, two separate studies conducted at the faculty of medicine in our university indicated that Cutaneous leishmaniasis and pulmonary tuberculosis also resulted in carcinogenesis and played a role in malignancies [6,7].

These studies promoted the idea that other infections might also cause carcinogenesis. For this purpose, we investigated the carcinogenesis effect of brucellosis. Obviously, brucellosis still remains to be a significant public health problem all over the world as well as in our country. It is a zoonotic infection caused by *Brucella* spp, which are facultative intracellular bacteria [17]. Brucella survives and replicates for prolonged periods within host macrophages and thus, has the ability to produce chronic infections [18]. It is not exactly known how brucella challenges and escapes the intracellular lethal mechanism of phagocytic cells. But it is clear that bactericidal functions including phagolysosomal fusion, neutrophile degranulation and oxidative burst in phagocytic cells are inhibited during brucella infection. The host defense system increases free radical production and antioxidant depletion in order to overcome the brucella infection [19,20]. Overproduction of reactive nitrogen species (RNS) and reactive oxygen species (ROS) is associated with host-mediated defense mechanisms, immunomodulation and autotoxicity that occur during microbial infection such as brucellosis and inflammation in certain cells [21, 22]. ROS and RNS are produced primarily to attack invading infectious agents. However, excess amounts of these reactive species in inflamed tissues can induce oxidative stress and result in the formation of oxidative and nitrative DNA lesions, such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [5].

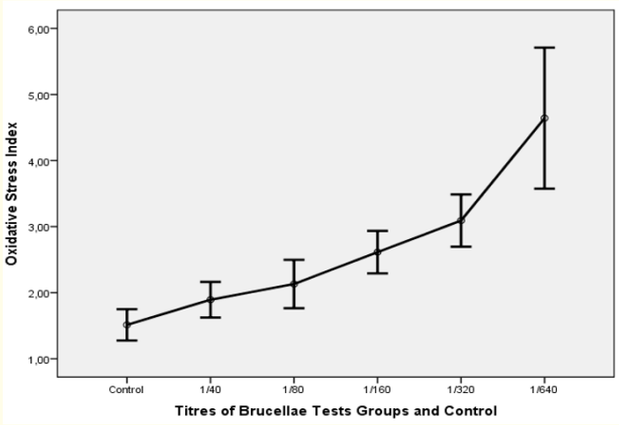
Previous studies demonstrated that decline in TAC and total thiol and increase in TOS, OSI and lipid peroxides (LOOH) result in DNA damage [7,23].

In our study, we compared the changes within the control group and brucellosis beginning from the lowest titer (1/40) to the highest titer (1/640). It was realized that compared to the control group, the total thiol (μmol/L) and TAC (mmolTrolox Equiv./L) level, which were anti-oxidant, declined as the brucella SAT increased from low titer to high titer. The declines were found to gain statistical significance at 1/160 and 1/320 titers, respectively ($P < 0.001$). In contrast to the anti-oxidant, it was determined that compared to the control group, lipid hydroperoxide (μmol H2O2 equiv./L), the molecule level of TOS (μmol H2O2 Equiv./L) and OSI rate, which took part in oxidative stress, increased as the brucella SAT increased from low titer to high titer. These changes were found to gain statistical significance at 1/640, 1/320 and 1/160 titers, respectively ($P < 0.001$). The changes were clearly demonstrated in the Table 2 and graphs (1-3). While molecules similar to the ones in our study were investigated in some of the previous studies, other oxidative molecules performing the same function were additionally examined in some other studies [2,6-10]. In order to kill the brucella microorganism, the active oxidative mechanism has begun to destroy the body cells apart from the pathogen microorganism.

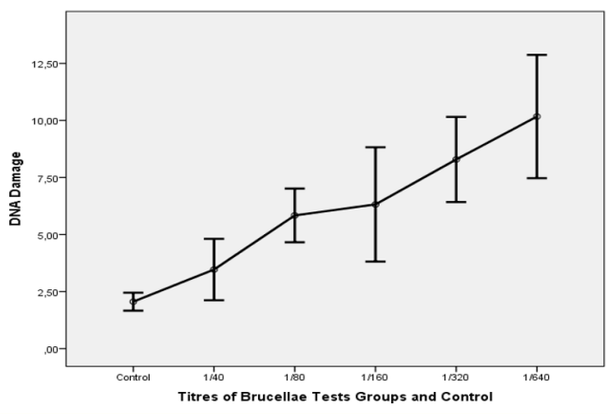
It is well-known that exposure of cells to oxidative stress leads to acute cellular metabolic disinfection. As a result of this, membrane lipid peroxidation, nicotinamide nucleotides depletion, intracellular free Ca+2 ions rise, cytoskeletal disruption and finally, DNA damage occur [24]. It is well known that accumulation of DNA damage and additionally DNA damage repair defect which develops with time can lead to gene modification in cells that may be mutagenic or carcinogenic [25,26]. As shown in the Table 2 and graphs (1-3), our study pinpointed that the increase in 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) level, which indicated DNA damage, signified the progress to mutation and carcinogenesis. Both the decrease in anti-oxidative level and the increase in oxidative stress and DNA damage began to gain statistical significance as the titer increased ($P < 0.001$). There was a significantly positive correlation between serum brucella SAT and DNA damage, TOS, OSI, LOOH levels (rho = 0.796, 0.810, 0.389, 0.717). And there was a significantly negative correlation between serum brucella SAT and TAC, SH levels (rho = -0.540, -0.503).



Graph 1: As the Brucella SAT titer increases, the total thiol decreases



Graph 2: As the Brucella SAT titer increases, OSI increases, too.



Graph 3: As the Brucella SAT titer increases, DNA damage increases, too.

In our study, it was determined that in parallel with the brucella SAT titer, oxidative stress and DNA damage, which would lead to mutation and carcinogenesis, also increased. In such patients, progress to carcinogenesis should be prevented through early diagnosis and treatment.

Based on the findings of our study, we consider that other chronic infections (such as extra pulmonary tb, osteomyelitis etc.) and inflammatory diseases (such as rheumatoid arthritis) should also be investigated due to their possibility to form a risk for mutation and carcinogenesis.

Conclusion

It was observed that as the SAT titer increased in brucellosis, oxidative stress and DNA damage also increased in relation to it, whereas the anti-oxidative capacity diminished. It was concluded that this damage might lead to brucellosis-related carcinogenesis. Therefore, one may argue that host cells might be prevented to proceed to carcinogenesis by hindering their exposure to oxidative stress through early treatment. Our study is the first one conducted on this topic and needs to be supported by prospective studies.

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No.

Conflicts of Interest

There are no conflicts of interest.

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