



## Alterations of Plasma Hecpidin, Homocysteine and Malondialdehyde in Vaccinated (S19 and RB 51 Vaccines) and Experimentally-Induced Brucellosis by *Brucella mellitensis* and *Brucella abortus* in Rat

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### ABSTRACT

In present study, we aimed to evaluate whether some plasma parameters are altered after intraperitoneal (i.p) injection of brucellosis vaccines and two types of *Brucella* species (*Brucella mellitensis* and *Brucella abortus*) in rat. Brucellosis is known to be as the common zoonotic disease. For this purpose, thirty male rats were assigned into five groups and *Brucella mellitensis*, *Brucella abortus* along with RB 51 and S19 vaccines were injected (i.p) to four groups and control ones received distilled water. After three months, plasma malondialdehyde (MDA), hepcidin (Hep) and homocysteine (Hcy) were measured in infected and vaccines-injected cases. The results indicate increase of MDA, Hep and Hcy in infected group rather than control ones ( $p \leq 0.01$ ). In respect of vaccines-injected group, increase of Hep along with no significant changes in others were revealed rather than control ones ( $p \leq 0.01$ ). In conclusion, the altered plasma parameters demonstrated the impact of *Brucella* species and those vaccines upon these parameters in rat and it seems more investigation needs to detect the possible biochemical marker among them.

**Keywords:** Plasma parameters, Brucellosis, Vaccines, rat

### INTRODUCTION

Brucellosis is the one of most common zoonosis disease in the world, accounting for an annual occurrence of more than 500,000 cases caused by a number of *Brucella* species [1] and Iran is still as the one of endemic areas. *Brucella* is a facultative intracellular, gram negative bacterium. Following infection through penetration of the mucosal epithelium, the bacteria migrate either free or within phagocytic cells to lymph nodes from where they may spread to any organ system via infected macrophages. Although its mortality is low, brucellosis is a serious disease that may affect multiple organ systems and cause various debilitating complications [2]. Several studies have explained brucellosis related serum parameters alterations such as trace elements, liver function and lipid profiles, but the wide varieties of them have not been determined till now [2,3]. Hence, this is the first work evaluation of clinical pathologic studies of brucellosis and its vaccines in rat.

Hcy, as a sulfur-containing amino acid, is generated from the intracellular demethylation of methionine. It is involved in endothelial cell damage in experimental animals and cardiovascular diseases in human. Hyperhomocysteinemia participates in oxidative stress occurrence and plays major role in pathological effects, which has been demonstrated as a mechanism involved in the formation of anaemia. Moreover, hyperhomocysteinemia promotes oxidant damage to vascular cells by several mechanisms: auto-oxidation, elevated production of ROS in platelets and homocysteine thiolactone interaction [4,5].

Free radicals (FRs) and Reactive Oxygen Species (ROS), predominantly oxyradicals, e. g. superoxide ( $O_2^-$ ), hydroxyl ion ( $OH^\cdot$ ), are continually produced during metabolic processes. As a result of the excessive generation of FRs, oxidative stress overwhelms the antioxidants available (redox imbalance) and stimulates some reactions causing cellular damage or cell death. Oxidative stress impresses on cell membrane polyunsaturated fatty acids - contained lipids and commences lipid peroxidation which is used as determinant of oxidative stress and cellular injury indicator. One of the end products of lipid peroxidation with low-molecular-weight is MDA. It is the most abundant and reliable biomarker to measure the degree of lipid peroxidation and the levels of free radicals indirectly [6-8].

Hepcidin (Hep) as the 25-amino-acid peptide is mainly synthesized in the liver. It is the essential hormone in regulating iron homeostasis and hepatic secretion of Hep is associated with response to iron overload. At the molecular level, Hep mechanism in iron homeostasis is related to inhibition of iron efflux from enterocytes, macrophages and hepatocytes into the plasma by inducing internalization and degradation of the iron exporter ferroportin in these cells [9,10]. Iron is an essential component of hemoglobin and myoglobin and it involves in the main structure of many enzymes during redox reactions and energy metabolism. Low levels of iron in the circulation may cause severe dysfunctions (e.g., anemia, hypoxia) while iron overload may be toxic because of its ability to generate reactive oxygen species. Excessive dietary iron uptake may cause iron deposition in many vital organs, including the liver, heart, skin, and especially pancreas [11-13]. Iron displays important interactions with other essential micro elements such as zinc and copper, showing competitive inhibitory effect in their transport and bioavailability.

To our knowledge, no assessment has been performed to investigate some biochemical parameters in brucellosis and vaccines-injected rats. Hence, this is the first work evaluate above-mentioned ones.

## MATERIALS AND METHODS

### Preparation of *Brucella mellitensis* and *Brucella abortus* suspension

For preparation of those suspensions, the fresh culture colonies were transferred to Muler-Hilton Broth and then its turbidity was regulated through 1Macfarland tube (approximately  $2 \times 10^8$  bacteria/ml).

### Procedure of study

In this trial, 30 male rats ( $45 \pm 4g$ ) assigned into five groups in special cages under standard hygienic conditions and were allowed to use water and standard pellet *ad libitum* and 12:12 h light: dark at temperature  $21-25^\circ C$  with 39% humidity. After 10 days of acclimatizing 0.5 ml of prepared bacterial suspensions injected intraperitoneal (i.p) to two groups (infected group) and the other two groups received 0.1 ml vaccines (RB51 and S19) and finally the distilled water was injected to control ones (6 rats). Three months later, all of them were anesthetized (by sodium pentobarbital, 60 mg/kg, i.p) and blood samples were collected from the inferior vena cava and then transferred to heparinized tubes and centrifuged at 5000 RPM for 10 minutes at  $4^\circ C$  and prepared plasma were frozen in  $-25^\circ C$  until analysis.

### Biochemistry parameters measurement

The ELISA kit (Bioassay Technology Laboratory Co, China) was utilized for plasma Hep concentration. Plasma MDA concentration was measured by Satoh method. Plasma Fe, Zn and Cu along with Hcy were determined by Spekol 1500 spectrophotometric device (Parsazmoon Co kits, Tehran, Iran).

## RESULTS

Alterations of all parameters have been denoted in Table (1). The results indicate significant increase ( $p < 0.01$ ) in all parameters in infected group in comparison with the healthy ones.

**Table 1. Alterations of some plasma parameters in infected groups compared with control ones**

Parameters	Control Group	<i>B.mellitensis</i> Group	<i>B.abortus</i> Group
Hcy (nmol/l)	5.40 ± 1.63	15.25 ± 2.71 <sup>†</sup>	12.58 ± 1.52 <sup>†</sup>
Hep (pg/ml)	137.38±22.46	350.10±67.56 <sup>†</sup>	312.23 ± 57.5 <sup>†</sup>
MDA (nmol/l)	4.53 ± 1.49	7.77 ± 1.25 <sup>†</sup>	8.46 ± 1.06 <sup>†</sup>
Fe (µg/dl)	37.68 ± 7.63	16.80 ± 1.85 <sup>†</sup>	21.18 ± 7.52 <sup>†</sup>
Cu (µg/dl)	55.15 ± 6.40	103.60 ± 14.75 <sup>†</sup>	89.10 ± 10.40 <sup>†</sup>
Zn (µg/dl)	66.35 ± 5.78	33.00 ± 4.19 <sup>†</sup>	36.90 ± 11.97 <sup>†</sup>

Data are expressed as mean ± standard deviation. † Significantly different from the control group (P<0.05)

**Table 1: Alterations of some plasma parameters in vaccinated group compared with control ones**

Parameters	Control Group	<i>S19</i> Group	<i>RB51</i> Group
Hcy (nmol/l)	5.40 ± 1.63	6.85 ± 1.18	5.97 ± 2.22
Hep (pg/ml)	137.38±22.46	198.62±20.67 <sup>†</sup>	185.32± 19.53 <sup>†</sup>
MDA (nmol/l)	4.53 ± 1.49	4.89 ± 1.04	4.72 ± 2.08
Fe (µg/dl)	37.68 ± 7.63	33.00 ± 6.23	33.75 ± 7.26
Cu (µg/dl)	55.15 ± 6.40	55.30 ± 4.43	64.82 ± 11.68
Zn (µg/dl)	66.35 ± 5.78	54.52 ± 6.78 <sup>†</sup>	53.55 ± 4.69 <sup>†</sup>

Data are expressed as mean ± standard deviation. † Significantly different from the control group (P<0.01)

## DISCUSSION

Plasma Hep was high in infected and vaccinated group than control ones. During infection, the Hep synthesis and secretion is induced for lowering iron bioavailability [14]. Several studies have been reported in respect of Hep and its roles in experimental infection and inflammation models. There are few studies which evaluate Hep levels in patients with infectious diseases [15,16]. The serum Hep elevation has been reported in pneumonia, sepsis and pyelonephritis and IL-6 induces Hep increase in various diseases. Yilmaz et al in 2014 revealed increased level of Hep in brucellosis patients and reported its possible biomarker in respect of brucellosis detection which is consistent with present study. Hep is positively modulated by elevated circulating iron and inflammatory cytokines, and therefore, Hep decreases ferroportin and availability of iron. It is known that Hep plays a fundamental role in innate immunity by decreasing bioavailability of iron for micro-organism utilization and by its direct antimicrobial effect [17,18]. Hence, it is possible due to inhibitory effects of physiologic system, Hep expression is elevated for decreasing of *Brucella* proliferation through decline of iron bioavailability and/or *Brucella* vaccines induce Hep expression through unknown mechanisms.

Our results in respect of Cu levels in plasma showed an increase in infected group (*B.mellitensis* and *B.abortus*) than control ones. In contrast, no significant changes were reported in vaccines-injected group compared control ones. Perhaps, high level of Cu may be attributed to infection, because high serum copper concentration has been reported in inflammation and infectious diseases in animals and human. Significant decrease of plasma Zn has been revealed in infected and vaccinated group. Our results are in accordance to those reported by Cesur et al. [19] and Mobaieen et al. [2] in brucellosis patients.

Elevated MDA was determined in infected rat not in vaccines-injected ones rather than control group. Reactive oxygen species (ROSs) involve as an important section of cellular immune response in killing of micro-organisms by leukocytes. Several studies have demonstrated increased involuntary production of ROSs in infections [7]. On the base of our knowledge, Kilic et al.[8] and Sherefhanoglu et al. [6] revealed increased levels of MDA in brucellosis patients compared with healthy ones which is in consistent with our study. In present study, MDA levels were significantly higher in infected group compared to control ones. It is speculated that this increase might be a result of an elevation of lipid peroxidation in affected tissues of infected group.

In respect of plasma Hcy, significant increase was showed in infected group rather than control ones. We could not find relevant studies regarding Hcy levels in brucellosis ones. Tamura et al [5] reported elevated levels of Hcy in *H pylori* infected group and related to low concentration of folic acid and vitamin B12. Azimzadeh [20] reported high concentration of Hcy in leptospirosis cattles in compared to healthy ones which all is in accordance with our study. It is possible that mal-absorption of those vitamins in infected ones is occurred whereby trans-methylation is disturbed and finally Hcy can't be converted to methionine.

In conclusion, present study suggest that experimental brucellosis and vaccinated ones play essential effects on alterations of some plasma parameters and it seems that medical assessment needs to determine possible potential biomarker among them.

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