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A Study on the Relationship between Heat Shock Proteins and Serum Biochemical Parameters in Clinically Healthy Bactrian Camels

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Abstract

This study was undertaken to investigate the HSPs levels in serum of different ages and sexes of clinically healthy Bactrian camels and their correlation with some biochemical parameters. Twenty-six two-humped adult camels in two sexes (male=18 and female=8), aged between 2 and 11 years old (2-4 years old=8, 5-8 years old=9, and 9-11 years old=9), were chosen for this study. Serum levels of HSP 30, HSP 40, HSP 70, and HSP 90 were measured by a quantitative sandwich enzyme immunoassay using commercial camel-specific kits. There were no significant differences in the concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in two sexes and different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*). However, there was a significant difference in the concentration of creatinine and sodium in two sexes and in the concentration of urea, albumin, and magnesium in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

Keywords: Heat shock proteins; Serum biochemical parameters; Bactrian camels

Introduction

Heat shock proteins, (HSPs) or stress proteins, are a large class of proteins which are expressed by prokaryote and eukaryote organisms. These proteins have a significant role in the protection of cellular homeostasis [1]. The HSP species belong to a large protein family consisting of constitutively expressed and inducible members, which are classified according to their molecular weight [2,3]. Thus, HSPs can be categorized and expressed in kDa: HSP15–30, HSP40, HSP60, HSP70, HSP90, and HSP100. Each HSP family includes several molecules, all sharing a similar primary structure and able to act analogous functions in various subcellular compartments [1]. Based on different studies, the production of HSPs as protective agents against harmful insults enhance transiently due to various stressors [4]. The HSPs are highly conserved proteins and act as molecular chaperons which confer thermotolerance and the protection of the cell to survive injury and oxidative stress [5]. Accordingly, environmental stresses, infection, normal physiological processes, and gene transfer can all promote HSPs level production [4]. *Camelus bactrianus*, also known as the Bactrian camel, inhabits parts of central Asia and western China. The distinguishing characteristic that sets Bactrian camels apart from dromedary camels is that they have two humps on their backs. There is no information about the HSPs in this species; besides, there has been no previous study about the relationships between these proteins and some biochemical factors (glucose, AST, LDL, cholesterol, phosphorus, iron, calcium, magnesium, total protein, albumin, globulin, urea) in Bactrian camels. Therefore, this study was undertaken to investigate the HSPs levels in the serum of different ages and sexes of clinically healthy Bactrian camels and their correlation with some biochemical parameters.

Materials and Methods

Animals and blood sampling

This study was conducted on female and male Iranian two-humped camels (*Camelus bactrianus*). The camels were reared at the Bactrian Camels Research Center in Ardabil province, Northwest Iran. Twenty-six adult camels in two sexes (male=18 and female=8), aged between 2 and 11 years old (2-4 years old=8, 5-8 years old=9, and 9-11 years old=9), were chosen for this study. The age of the animals was estimated using dental characteristics. All the animals were clinically healthy and free from internal and external parasites. The blood sample was collected into a 10-ml vacuum tube, chilled immediately after sampling, and transported to the laboratory within 1 h after the collection. Serum was harvested after the centrifugation at 750 g for 15 minutes, frozen, and stored at -21°C until analysis.

Animal ethics

All animal experiments were approved by the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). The recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes, were also followed.

Measurement of the parameters

Serum levels of HSP 30, HSP 40, HSP 70, and HSP 90 were measured by a quantitative sandwich enzyme immunoassay using commercial camel-specific kits (Shanghai Crystal Day Biotech, Shanghai, China). The sensitivity of HSP 30 kit was 0.021 ng/ml. The intra-assay precision and inter-assay precision of HSP 30 kit were CV<8% and CV<10%, respectively. The sensitivity of HSP 40 kit was 0.023 ng/ml. The intra-assay precision and inter-assay precision of HSP 40 kit were CV<8% and CV<10%, respectively. The sensitivity of HSP 70 kit was 0. 24 ng/ml. The intra-assay precision and inter-assay precision of HSP 70 kit were CV<8% and CV<10%, respectively. The sensitivity of HSP 90 kit was 0. 14 ng/ml. The intra-assay precision and inter-assay precision of HSP 90 kit were CV<8% and CV<10%, respectively.

Serum biochemical parameters including blood urea nitrogen

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(BUN), creatinine, glucose, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, total protein, albumin, AST and ALT were measured using standard methods and commercial kits (Pars Azmoon Co., Tehran, Iran), and a biochemical auto analyzer (Alpha Classic AT^{++}, Sanjesh, Iran). The globulin values were calculated by subtracting albumin values from the total protein. To evaluate the serum concentration of elements, digestion of serum was performed by a mixture of perchloric acid and nitric acid (3:7 ratio respectively). Then, Ca, P, Mg and Fe were measured using an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan). Serum concentration of sodium and potassium were measured using an flame photometer apparatus (Fater Electron Company, Tehran, Iran).

Statistical analysis

Statistical analysis was performed using SPSS (Version 12.0; SPSS, Chicago, USA). Two sample t-test was used to detect differences in the parameters between the two sexes. Correlations were analyzed by Pearson's correlation tests and analysis of variance (ANOVA) tests was used to compare the serum concentrations of the measured factors between different age groups. Differences were considered significant at $P<0.05$.

Results

The concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in two sexes of clinically healthy Bactrian camel (*Camelus bactrianus*) is shown in Table 1. There were no significant differences in the concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in two sexes of clinically healthy Bactrian camels (*Camelus bactrianus*).

The concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*) is shown in Table 2. There were no significant differences in the concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

The concentration of serum biochemical parameters in two sexes of clinically healthy Bactrian camels (*Camelus bactrianus*) is shown in Table 3. There was a significant difference in the concentration of creatinine and sodium in two sexes of clinically healthy Bactrian camels (*Camelus bactrianus*).

The concentration of serum biochemical parameters in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*) is shown in Table 4. There was a significant difference in the concentration of urea, albumin, and magnesium in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

In addition, we found a statistically significant positive correlation between HSP90 and glucose ($r=0.862$; $P<0.01$), LDL ($r=0.737$; $P<0.05$), phosphorus ($r=0.631$; $P<0.01$), total protein ($r=0.634$; $P<0.05$), iron ($r=0.914$; $P<0.01$), and cholesterol ($r=0.847$; $P<0.01$); HSP40 and glucose ($r=0.568$; $P<0.05$), calcium ($r=0.679$; $P<0.05$), and AST ($r=0.586$; $P<0.05$); HSP70 and glucose ($r=0.515$; $P<0.05$), urea ($r=0.424$; $P<0.05$), and cholesterol ($r=0.471$; $P<0.05$); HSP30 and glucose ($r=0.676$; $P<0.05$), calcium ($r=0.856$; $P<0.01$), albumin ($r=0.826$; $P<0.05$), and magnesium ($r=0.471$; $P<0.05$).

Discussion

HSPs can induce the tolerance of environment and cellular

Factor / Sex	HSP 90(ng/ml)	HSP 40(ng/ml)	HSP 70(ng/ml)	HSP 30(ng/ml)
Male (n=18)	14.76 ± 0.70^{a}	3.04 ± 0.23^{a}	23.78 ± 1.77^{a}	2.25 ± 0.11^{a}
Female (n=8)	15.60 ± 0.84^{a}	3.60 ± 0.43^{a}	19.13 ± 1.5^{a}	2.40 ± 0.18^{a}
Different letters in each column demonstrate significant differences (P<0.05).				

Table 1: The concentration of heat shock proteins in two sexes of clinically healthy Bactrian camels (*Camelus bactrianus*).

Factor / Age (year)	HSP 90(ng/ml)	HSP 40(ng/ml)	HSP 70(ng/ml)	HSP 30(ng/ml)
2-4 (n= 8)	14.62 ± 0.91^{a}	2.80 ± 0.32^{a}	20.18 ± 0.81^{a}	2.24 ± 0.24^{a}
5-8 (n= 9)	15.24 ± 1.18^{a}	3.34 ± 0.35^{a}	27.36 ± 3.07^{a}	2.1 ± 0.07^{a}
9-11 (n= 9)	15.35 ± 0.78^{a}	3.45 ± 0.41^{a}	19.16 ± 1.40^{a}	2.45 ± 0.17^{a}
Different letters in each column demonstrate significant differences (P<0.05).				

Table 2: The concentration of heat shock proteins in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

Factor / Sex	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	TG (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)
Male (n=18)	70.80 ± 4.29^{a}	63.66 ± 4.54^{a}	1.28 ± 0.043^{a}	26.41 ± 2.13^{a}	44.11 ± 2.47^{a}	9.47 ± 0.44^{a}
Female (n=8)	65.25 ± 5.34^{a}	42.63 ± 5.23^{a}	1.76 ± 0.39^{b}	29.87 ± 4.58^{a}	38.75 ± 3.36^{a}	10.86 ± 0.46^{a}
Different letters in each column demonstrate significant differences (P<0.05).						
Factor / Sex	**LDL (mg/dl)**	**TP(g/dl)**	**ALB (g/dl)**	**GLB (g/dl)**	**AST (U/L)**	**ALT(U/L)**
Male (n=18)	32.69 ± 1.98^{a}	6.16 ± 0.19^{a}	4.76 ± 0.14^{a}	1.39 ± 0.13^{a}	40.33 ± 0.42^{a}	39.43 ± 0.52^{a}
Female (n=8)	24.77 ± 1.95^{a}	5.89 ± 0.41^{a}	4.62 ± 0.27^{a}	1.27 ± 0.19^{a}	39.51 ± 0.57^{a}	38.79 ± 0.80^{a}
Different letters in each column demonstrate significant differences (P<0.05).						
Factor / Sex	**Na (mmol/l)**	**K (mmol/l)**	**Ca (mg/dl)**	**P (mg/dl)**	**Mg (mg/dl)**	**Fe (µg/dl)**
Male (n=18)	128.67 ± 3.15^{a}	6.73 ± 0.34^{a}	11.49 ± 0.27^{a}	8.27 ± 0.43^{a}	2.86 ± 0.07^{a}	70.6 ± 4.40^{a}
Female (n=8)	134.50 ± 8.64^{b}	7.60 ± 0.52^{a}	12.01 ± 0.57^{a}	6.47 ± 0.52^{a}	2.54 ± 0.12^{a}	58.68 ± 7.93^{a}
Different letters in each column demonstrate significant differences (P<0.05).						

Table 3: Serum biochemical parameters in two sexes of clinically healthy Bactrian camels (*Camelus bactrianus*).

Factor / Age (year)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	TG (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)
2-4 (n= 8)	66.68 ± 3.95[a]	80.68 ± 3.77[a]	1.36 ± 0.06[a]	30.50 ± 3.25[a]	39.93 ± 3.99[a]	9.48 ± 0.93[a]
5-8 (n= 9)	74.66 ± 7.89[a]	49.66 ± 4.30[b]	1.24 ± 0.05[a]	23.10 ± 2.79[a]	48.83 ± 2.78[a]	9.51 ± 0.40[a]
9-11 (n= 9)	65.66 ± 4.73[a]	43.84 ± 4.76[b]	1.69 ± 0.35[a]	29.16 ± 4.1[a]	38.33 ± 2.99[a]	10.65 ± 0.46[a]
Different letters in each column demonstrate significant differences (P<0.05).						
Factor / Age (year)	**LDL (mg/dl)**	**TP (g/dl)**	**ALB (g/dl)**	**GLB (g/dl)**	**AST (U/L)**	**ALT(U/L)**
2-4 (n= 8)	31.12 ± 3.57[a]	6.58 ± 0.26[a]	5.23 ± 0.19[a]	1.34 ± 0.12[a]	40.23 ± 0.68[a]	39.07 ± 0.92[a]
5-8 (n= 9)	35.38 ± 1.94[ab]	5.92 ± 0.23[a]	4.46 ± 0.12[b]	1.46 ± 0.25[a]	40.40 ± 0.63[a]	39.60 ± 0.67[a]
9-11 (n= 9)	24.35 ± 1.77[ac]	5.79 ± 0.37[a]	4.52 ± 0.26[b]	1.26 ± 0.17[a]	39.63 ± 0.51[a]	39.02 ± 0.74[a]
Different letters in each column demonstrate significant differences (P<0.05).						
Factor / Age (year)	**Na (mmol/l)**	**K (mmol/l)**	**Ca (mg/dl)**	**P (mg/dl)**	**Mg (mg/dl)**	**Fe (µg/dl)**
2-4 (n= 8)	125.88 ± 3.39[a]	6.12 ± 0.52[a]	11.58 ± 0.31[a]	8.22 ± 0.68[a]	3.13 ± 0.05[a]	68.62 ± 2.49[a]
5-8 (n= 9)	132.33 ± 5.41[a]	7.32 ± 0.45[a]	11.57 ± 0.45[a]	8.62 ± 0.57[ab]	2.66 ± 0.06[b]	74.77 ± 8.33[a]
9-11 (n= 9)	132.67 ± 7.83[a]	7.46 ± 0.48[a]	11.78 ± 0.55[a]	6.36 ± 0.47[ac]	2.54 ± 0.11[ac]	57.77 ± 7.06[a]
Different letters in each column demonstrate significant differences (P<0.05).						

Table 4: Serum biochemical parameters in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

stresses in mammals. The HSPs are highly conserved proteins which get activated by heat and other stressors [6]. In particular, camels that are exposed to various stresses are naturally equipped with these proteins to deal with different environmental stresses [7]. The HSP70 is one of the most abundant HSP families playing a crucial role in the environmental stress [8]. Like other mammalian species, camels are also known to have HSP70 family genes to manage stress conditions [9]. Members of the HSP70 family are well recognized to protect prokaryotic as well as mammalian cells from thermal stress or hypoxic stresses [10]. Considering the fact that only a few studies have been done about the HSPs in camels, we tried to investigate the various HSPs in Bactrian camels.

In the present study, no significant differences were found in the concentration of heat shock proteins (HSP 90, HSP40, HSP70 and HSP 30) in two sexes and different age groups of clinically healthy Bactrian camels. Thus, it can be concluded that Bactrian camels in different sexes and ages may have similar HSP responses. It is proved that urine heat shock protein (HSP 72) can increase in acute kidney injury [11]. HSP70 can be used as a useful marker in human kidney disease [12,13]. In our study, we found some correlation between HSP 70 and urea, so it can be suggested that the HSP70 in blood may also increase in camel prerenal disease.

In human studies, it is suggested that a high glucose concentration can increase HSP70 [14]. This can happen as a result of the oxidative stress following hyperglycemia which can lead to an increase in HSPs [15]. A similar mechanism to this can occur in Bactrian camels and that could justify the positive correlation between HSPs and glucose. In our study, we observed some correlation between lipids and HSPs. Kuan et al. [16] also demonstrated *in vivo* that HSP90 inhibition reduced lipids levels in mouse livers. We can suggest that any changes in serum lipids can induce stress and increase HSPs.

Conclusion

There were no significant differences in the concentration of heat shock proteins (HSP 90, HSP 40, HSP70, and HSP 30) in two sexes and different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*). However, there was a significant difference in the concentration of creatinine and sodium in two sexes and in the concentration of urea, albumin, and magnesium in different age groups of clinically healthy Bactrian camels (*Camelus bactrianus*).

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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