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Comparative study on serum protein concentration and electrophoretic pattern in clinically healthy gazelles

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ABSTRACT

Screening of electrophoretic pattern of serum protein is crucial for the initial evaluation of numerous clinical conditions. The present study aimed to investigate and compare the normal serum protein electrophoretic pattern in three gazelle species which included sand gazelle (*Gazella gazella*), mountain gazelle (*Gazella subgutterosa*) and Erlanger's gazelle (*Gazella erlangeri*). Significant ($P \leq 0.05$) interspecies differences were noticed in A/G ratio and in α-1, β and ɣ globulins fractions using agarose gel electrophoresis. Significant ($P \leq 0.05$) differences between males of the three species were encountered in α-1, α- 2 and β globulins fractions, while significant differences in A/G ratio, ɣ , α-1, α-2 and β globulins fractions were observed between females of the three species. Significance ($P \leq 0.05$) intersex differences were observed for albumin in sand gazelle and for α-2 and β globulins fractions in mountain gazelle. The results obtained from the current study could provide a baseline references and could be used for diagnostic purposes.

Key words: Electrophoresis, Gazelle, Gender, Serum protein.

Serum electrophoresis is an easy and inexpensive mothed of separating protein based on their net charge size and shape. Hundreds of different proteins circulate in blood and their concentrations are of clinical value. Investigation and information on serum protein in animals are scarce and available only for few species (Brown *et al*., 1989). It is well known that the distribution and concentration of blood protein is affected by many diseases such as brucellosis (Wang and Wu, 2017). However, as blood protein fraction varies with species, the determination of normal electrophoretic pattern of blood protein for each species is essential (Ahamdi-Hamedani *et al*., 2014). Previous reports have described protein electrophoresis pattern as diagnostic tool in conditions affecting serum protein concentration (Esmaeilnejad *et al*., 2012; Csilla *et al*., 2013). In addition, protein electrophoretic pattern have been reported in clinically healthy ruminants (Alberghina *et al*., 2010; Piccione *et al*., 2011, 2012; Nagy *et al*., 2015). However, research on protein electrophoresis in clinically healthy wild animals or its use as diagnostic tool is scares (Zhou *et al*., 2009. Hussein *et al*., 2010; Xiaoyun and Zhigang, 2012). Therefore, this study was designed to determine and compare the normal serum protein electrophoretic pattern and the absolute relative concentration of serum protein fractions in three clinically healthy gazelle species using agarose gel electrophoresis.

The present study was conducted using 22 clinically health sand gazelle (Idmi, 11 males and 11 females), 21 mountain gazelle (Reem, 10 males and 11 females) and 12 Erlanger's gazelle (8 males and 4 females). The animals were kept in small groups pens at King Khalid Wildlife Research Centre (KKWRC) in Thumamah (25° 30″ N, 46° 30″ E), Saudi Arabia. During this experiment gazelles were fed on dry alfalfa and concentrate (16% protein) and had free access to fresh clean tap water and mineral salt licks. During blood collection, gazelles were handled manually using drive-in boma (Pienaar, 1973). Blood samples were collected through jugular vein puncture into plane vacutainers. Then, the blood samples were allowed to clot at room temperature and centrifuged at 3000x g for 15 min to separate the serum. Separated serum samples were stored at -20°C till biochemical analysis. All procedures described in this experiment were approved by the Research Ethics Committee at the King Saud University.

The concentration of total protein in serum samples was determined by spectrophotometric method using commercial kit (Randox Company - UK). Serum proteins were separated by agarose gel electrophoresis using Helena SAS MX-electrophoresis (Helena Bio system Europe, standard UK). The electrophoresis was performed at 80v for 14 min. Human control sera (Kemtrol Serum Control - Normal and Abnormal;

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Helena laboratories) were used in each test. After migration the gel were fixed with acid, stained and dried in oven at 37 for 40 min. Electrophoretic gel were scanned and the serum protein fractions were visualized and displayed on densitometry system using Epson Expression 1680 pro scanner (Seiko Epson, Amsterdam, Netherland). The relative concentrations of protein fractions were determined and the absolute values of fractions were computed from total protein concentration.

Data analysis and statistical procedure

The obtained data were analyzed as a full factorial design in two factors (species and gender) using the PROC FACTEX procedure of statistical analysis system (SAS Inst., Inc., Cary, NC). Data were subjected to ANOVA using α = 0.05. Means showing significant differences in ANOVA were tested using the PDIFF option. The probability value denoting statistical significance was P ≤ 0.05. Means and their SE are presented in Tables.

Serum protein electrophoresis for the three gazelle species exhibited four fractions (albumin, α-, β- and ɣ-globulins). Moreover, α- globulin band was further sub-divided into α1 and α-2 globulins fractions. Significant (P ≤ 0.05) differences in A/G ratio, ɣ globulin, α-1, α- 2 and β globulin fractions were noticed between gazelle species (Table 1). On the other hand, significant (P ≤ 0.05) differences in A/G ratio, ɣ globulin, α-1, α-2 and β globulins fractions were noticed between females of the three gazelle species (Table 2), while a significant (P ≤ 0.05) differences in α-1, α-2 and β globulin fractions were observed between males of the three gazelle species (Table 3).

Over all means of total protein values for Idmi and Reem gazelles presented in the current study resemble that reported for other gazelle's species (Hammer *et al*., 2008; Mohammed *et al*., 2011; Ibanez *et al*., 2016). However, the herein reported values for serum total protein are lower than that reported for Dorcas gazelle (Agab *et al*., 2013; Babor *et al*., 2014), gazelle subgutterosa, Prezewaiki's and Tibetan gazelles (Askar *et al*., 2007; Zhou *et al*., 2009; Xiaoyun and Zhigang, 2012). While, the value of serum total protein for Erlanger's gazelle presented in this study is lower than that reported by Aljumaah and Hussein (2011) for the same species.

Although, the overall means of α, β and ɣ globulins presented herein for the three gazelle species is different from that reported for Dorcas gazelle, black buck (Antilope cervicapra), Prezewaiki's and Tibetan gazelles (Abaigar, 1993, Xiaoyun and Zhigang, 2012, Zhou *et al*., 2009), it is similar to that reported for Cuvier's gazelle (Ibanez *et al*., 2016), chital deer, (Chapple *et al*., 1991), red deer (Rosef *et al*., 2004) and Arabian Oryx (Hussein *et al*., 2010).

Table 1: Concentration of serum protein fractions (means ± SE) of gazelle species.

Parameter	Gazelle Species			*P*
	Idmi (22)	Erlangeri (12)	Reem (21)	
Total protein (g/dL)	5.28±0.19	5.27±0.28	5.01±0.17	NS
Albumin (g/dL)	2.74±0.11	2.68±0.14	2.84±0.11	NS
Globulin (g/dL)	2.54±0.12^{ab}	2.59±0.18^{a}	2.17±0.10^{b}	<0.05
A/G ratio	1.12±0.07^{b}	1.07±0.06^{b}	1.32±0.07^{a}	<0.05
Alpha 1 (g/dL)	0.29±0.01^{b}	0.36±0.04^{a}	0.23±0.01^{c}	<0.05
Alpha 2 (g/dL)	0.63±0.08^{b}	0.84±0.05^{a}	0.34±0.02^{c}	<0.05
Beta (g/dL)	0.57±0.08^{ab}	0.56±0.05^{b}	0.71±0.03^{a}	<0.05
Gamma (g/dL)	1.06±0.10^{a}	0.75±0.05^{b}	0.84±0.06^{ab}	<0.05

^{a,b,c}Means within the same row with different superscripts are significantly different at P ≤ 0.05; A/G = Albumin/Globulin; Numbers between brackets refer to the number of animals used in the experiment.

Table 2: Concentration of serum protein fractions (means ± SE) in female gazelle species.

Parameter	Gazelle Species			*P*
	Idmi (11)	Erlangeri (8)	Reem (11)	
Total protein (g/dL)	5.32±0.23	5.28±0.40	5.32±0.29	NS
Albumin (g/dL)	2.69±0.09	2.71±0.21	3.13±0.16	NS
Globulin (g/dL)	2.64±0.19	2.57±0.20	2.19±0.16	NS
A/G	1.06±0.09^{b}	1.07±0.05^{b}	1.35±0.11^{a}	<0.05
Alpha 1 (g/dL)	0.27±0.01^{b}	0.38±0.04^{a}	0.21±0.02^{b}	<0.05
Alpha 2 (g/dL)	0.30±0.02^{b}	0.84±0.06^{a}	0.36±0.03^{b}	<0.05
Beta (g/dL)	0.91±0.05^{a}	0.56±0.04^{c}	0.73±0.04^{b}	<0.05
Gamma (g/dL)	1.14±0.15^{a}	0.69±0.05^{b}	0.81±0.7^{b}	<0.05

^{a,b,c}Means within the same row with different superscripts are significantly different at P ≤ 0.05; A/G = Albumin/Globulin ratio; Numbers between brackets refer to the number of animals used in the experiment.

Table 3: Concentration of serum protein fractions (means ± SE) in male gazelle species

Parameter	Gazelle Species			*P*
	Idmi (11)	Erlangeri (4)	Reem (10)	
Total protein (g/dL)	5.25±0.30	5.25±0.31	4.70±0.12	NS
Albumin (g/dL)	2.80±0.20	2.62±0.09	2.56±0.09	NS
Globulin (g/dL)	2.45±0.17	2.63±0.38	2.15±0.13	NS
A/G	1.17±0.10	1.07±0.17	1.29±0.10	NS
Alpha 1 (g/dL)	0.30±0.02^{ab}	0.36±0.07^{a}	0.24±0.01^{b}	<0.05
Alpha 2 (g/dL)	0.93±0.06^{a}	0.85±0.12^{a}	0.33±0.02^{b}	<0.05
Beta (g/dL)	0.27±0.06^{b}	0.56±0.13^{a}	0.70±0.03^{a}	<0.05
Gamma (g/dL)	0.99±0.14	0.88±0.09	0.88±0.10	NS

^{a,b}Means within the same row with different superscripts are significantly different at P ≤ 0.05; A/G = Albumin/Globulin ratio; Numbers between brackets refer to the number of animals used in the experiment.

Table 4: Within species intersex variations in the concentration of serum protein fractions (means ± SE) of gazelle species.

Parameter	Gazelle Species						*P*
	Idmi f (11)	Idmi m (11)	Erlangeri f (8)	Erlangeri m (4)	Reem f (10)	Reem m (11)	
TP (g/dL)	5.32±0.23	5.25±0.30	5.28±0.40	5.25±0.31	5.32±0.29	4.70±0.12	NS
Albumin (g/dL)	2.69±0.09	2.80±0.20	2.71±0.21	2.62±0.09	3.13±0.16^{a}	2.56±0.09^{b}	<0.05
Globulin (g/dL)	2.64±0.19	2.45±0.17	2.57±0.20	2.63±0.38	2.19±0.16	2.15±0.13	NS
A/G ratio	1.06±0.09	1.17±0.10	1.07±0.05	1.07±0.17	1.35±0.12	1.29±0.10	NS
Alpha 1 (g/dL)	0.27±0.01	0.30±0.02	0.38±0.04	0.36±0.07	0.21±0.02	0.24±0.01	NS
Alpha 2 (g/dL)	0.30±0.02^{b}	0.93±0.06^{a}	0.84±0.06	0.85±0.12	0.36±0.03	0.33±0.02	<0.05
Beta (g/dL)	0.91±0.05^{a}	0.27±0.06^{b}	0.56±0.04	0.56±0.13	0.73±0.04	0.70±0.03	<0.05
Gamma (g/dL)	1.14±0.15	0.99±0.14	0.69±0.05	0.88±0.09	0.82±0.07	0.88±0.10	NS

^{a,b}Means within the same row with different superscripts are significantly different at P ≤ 0.05; f = female; m = male; TP = Total protein; A/G = Albumin/Globulin; Numbers between brackets refer to the number of animals used in the experiments.

The results obtained concerning protein fractions were within the references range for Dama gazelle and Cuvier's gazelle (Abaigar, 1993, Ibanez *et al*., 2016), but higher than that reported for some wild ruminants (Peinado *et al*., 1999) and lower than that reported for domestic ruminants (Qureshi *et al*., 2016; Gupta *et al*., 2017). It has been reported that serum protein fraction display important differences among all domestic animals (Alberghina *et al*., 2010, Nagy *et al*., 2015). The observed difference between species in protein fractions could be due to electrophoretic mobility (Irfan, 1967). Therefore, it is essential to determine the normal electrophoretic pattern of each species.

In the current study we observed significant intersex differences (P ≤ 0.05) only for α-2 and β- globulins fractions in Idmi gazelle and for albumin in Reem gazelle (Table 4). However, no significant differences in serum protein fractions between male and female were observed in Erlanger's gazelle and for other protein fractions in Idmi and Reem gazelles. Similarly, Hussein *et al*. (2010) reported gender independent protein fractions in Arabian Oryx and chital deer.

CONCLUSION

The results obtained from the present study could provide baseline references for serum protein fractions in the studied gazelle species and could further be used as diagnostic tool.

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