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A preliminary study on physiological dynamism of various serum cardiac biomarkers in apparently healthy Sipli sheep of Pakistan

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ABSTRACT

Background: For sheep in tropical/subtropical areas of the world, the reported mortality and morbidity rate regarding cardiomyopathies is quite low (1%–2%), yet it has been elucidated that many sheep slaughtered for meat purposes in Pakistan have enlarged hearts with underlying cardiac abnormalities.

Aim: The present preliminary work is the first record of deducing normal reference intervals (RIs) for various serum cardiac biomarkers namely cardiac troponin I (cTnI), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase-myocardial band (CK-MB), sodium (Na^+), potassium (K^+), and total protein (TP), and their interrelationship in apparently healthy indigenous Sipli breed of sheep ($n = 141$) from Pakistan.

Methods: Apropos to aseptic blood collection and serum extraction, the attributes were deduced using commercially available kits. For the sake of analyses, the studied biomarkers were attributed as independent (Na^+, K^+, and TP) and dependent (LDH, ALT, AST, cTnI, and CK-MB) variables. The RIs were determined through Reference Value Advisor Version 2.1 as *per* gender and age keeping in view the guidelines provided by the American Society of Veterinary Pathology.

Results: The RIs for cardiac serum biomarkers included in the present study were generally within the reference range provided for sheep with slight variations which might be attributed to physiological adaptation to harsh desert climate. The Na^+ significantly ($p \leq 0.05$) predicted AST (adj. r-square = 0.976, 97% probability) and CK-MB (adj. r-square = 0.477, 47% probability). Similarly, K^+ significantly ($p \leq 0.05$) predicted LDH (adj. r-square = 0.626, 62% probability) and ALT (adj. r-square = 0.897, 89% probability). The TP had the highest adjusted r-square of 0.770 (77% probability) with AST; however, it was statistically non-significant ($p \geq 0.05$).

Conclusion: The results of the study present baseline data about these cardiac biomarkers which may be utilized for cardiac assessment of the sheep. It is recommended that the serum cardiac biomarkers ascertained in this study be studied in perspective to electrophysiology and ultrasonography of sheep heart for confirmed diagnosis/prognosis of cardiomyopathies.

Keywords: Sipli sheep, Cardiac troponins, Reference intervals.

Introduction

Biomarkers are specific and distinct biological indicators of various processes and conditions taking place in an organism that are indicative of a certain physiological or pathological event. For human medical sciences, extensive work has been conducted on ascertaining various cardiac biomarkers for an earlier diagnosis/prognosis of cardiomyopathies. Ultimately, creatinine kinase (along with its isoenzyme CK-myocardial band; CK-MB), lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST) have been validated as gold-standards for assessing cardiac health in humans (Siegel *et al.*, 2008). Finally, it has been elucidated that the levels of cardiac troponins (cTnI and cTnT) are far better indicators of cardiac health. Troponin, in fact, is a protein complex, is a part of the thin filament of heart muscles, and consists of three subunits, i.e., Troponin-I, Troponin-C, and Troponin-T (Azzazy and Christenson, 2002; Wells and Sleeper, 2008). Normally, its values are assessed along with those of CK-MB, LDH, AST, and ALT for better diagnostics/prognostics of cardiac myopathy.

Regarding veterinary medical sciences, increased levels of the aforementioned cardiac biomarkers in various cattle breeds and pet animals affected with various cardiac pathologies have been reported globally

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(Nikvand *et al.*, 2019; Sepulveda *et al.*, 2022). However, to the best of our knowledge, no such work on cardiac biomarkers has yet been conducted on indigenous livestock of Pakistan. Though for sheep, the mortality and morbidity rate regarding cardiomyopathies is much lower (1%–2%) (Válková *et al.*, 2024), it has been elucidated that many sheep slaughtered for meat purposes in Pakistan have enlarged hearts with underlying cardiac abnormalities (Z. Saeed, Personal Communication).

Pakistan has a temperate to tropical weather and harbors about 31.9 million heads of sheep belonging to 17 different breeds which probably originated from urial (*Ovis vignei*), the wild sheep of Afghanistan, Baluchistan, and Central Asia (Khan *et al.*, 2008). Sipli is a medium-sized, thin-tailed indigenous sheep breed of Pakistan with a relatively long tail. With an average body weight of 32.8 kg for males and 29.2 kg for females, it has a daily milk yield of 0.2–0.4 l and a fiber yield of approximately 5.6 kg *per* annum (Jaffar, 2011). It has a white body coat with white or light brown head/ears. Its head is medium sized and has a flat nose with ears reaching about 15 cm long. It is mostly reared for mutton and wool purposes by the nomadic herders of Bahawalpur, Bahawalnagar, and Rahim-Yar-Khan- the three cities lay in the middle of the Cholistan desert, (Southern Punjab) Pakistan. For this sheep breed, our laboratory has recently reported normal physiological reference intervals (RIs) for various serum biochemical attributes (Idris *et al.*, 2024), and a serum color chart for on-field estimation of hemoglobin and bilirubin (Idris *et al.*, 2023). The present preliminary work is the first record of deducing normal RIs for various serum cardiac biomarkers [cTnI, ALT, AST, LDH, CK-MB, Na^+, K^+, and total protein (TP)], and their interrelationship, in apparently healthy indigenous Sipli breed of sheep from Pakistan. This set of cardiac biomarkers is generally in vogue for assessing cardiac health both in human and veterinary medical diagnostics/prognostics. The results of the study cater a baseline data about these cardiac biomarkers which may be utilized for cardiac assessment of the sheep.

Materials and Methods

Geo-location of study

The present study was carried out simultaneously at the Livestock Farm, Faculty of Veterinary and Animal Sciences (FV and AS), The Islamia University of Bahawalpur (IUB), Pakistan, and Post-graduate Lab of Physiology, IUB. The climate of the Cholistan desert is arid and semi-arid tropical; the average temperature of the Cholistan desert is 28.33°C, average rainfall of the Cholistan desert is up to 180 mm (Farooq *et al.*, 2010).

Experimental animals

The Sipli sheep breed (n = 141) reared at the Livestock Farm of FV and AS, IUB, Pakistan under an intensive farming system was incorporated in the present study. The animals under study were grouped by gender and age (G1 = up till 1 year, G2 = from 1 to 2 years, G3 = above 2 years). The animals are sent for grazing early morning. In the evening the feeding of animals includes fresh-cut and chopped seasonal fodder along with a concentrate ration containing about 15% crude protein. In addition, maize silage and wheat straw are offered depending on need as and when required. The fresh clean drinking water remains available all the time. The animals have been assigned tag numbers to collect data.

Blood collection, processing, and analyses

Approximately 5 ml blood sample was collected from each experimental animal. Bleeding was conducted once with a total of 141 blood samples. The blood was collected aseptically from the jugular vein using a 5 ml disposable syringe in yellow-capped vacutainers containing silica and a polymer gel for serum separation. The vacutainers were centrifuged at 3,000 rpm for 15 minutes by a centrifuge machine (Centrifuge 800, China) for serum extraction. Serum was extracted in Eppendorf tubes which were transported in ice packs to the laboratory for further analysis. The serum cardiac biomarkers were assayed using commercial kits as follows and the optical density was taken through a spectrophotometer:

a. LDH: Bioactive Diagnostic Systems, CAT No. 104989993202, JTC, Germany, Sensitivity 25 U/l, Linearity up to 2,000 U/l, Repeatability 254.2 U/l.
b. AST: Bioactive Diagnostic Systems, CAT No. 104989993177, JTC, Germany, Sensitivity 3 U/l, Linearity up to 500 U/l, Repeatability 34.3 U/l.
c. CK-MB: Bioactive Diagnostic Systems, CAT No. 10498999399, JTC, Germany, Sensitivity 4.5 U/l, Linearity up to 1,000 U/l, Repeatability 33.2 U/l.
d. ALT: Martin Dow Specialties, Pakistan, CAT No. 104989993271, Sensitivity 23 U/l, Linearity up to 500 U/l, Repeatability 54.2 U/l.
e. TP: Bioactive Diagnostic Systems, CAT No. 104989993190, JTC, Germany, Sensitivity 0.17 g/dl, Linearity up to 15 g/dl, Repeatability 3.91 g/dl.
f. Na^+ and K^+: Bioactive Diagnostic Systems, CAT No. 104989993353, JTC, Germany.

cTnI determination

The cTnI was determined using Ichroma III Immunoassay Analyzer (Germany) with a kit by Boditech Med Incs, Korea, CAT No. INS14 having a sensitivity of 18.44 ng/ml, specificity of 97.1%, and linearity of up to 10.0 ng/ml. A chemiluminescence immunoassay (CLIA) platform was used for this purpose which offers high sensitivity and specificity for cTnI detection. In this method, a blood sample was processed through an automated CLIA system where cTnI binds to specific antibodies coated on magnetic particles. Upon binding, a chemiluminescent substrate was introduced, emitting light proportional to the cTnI concentration. This luminescent signal was quantified using a photomultiplier, with the intensity directly

correlating to the cTnI levels in the sample, allowing accurate and rapid determination.

Statistical analyses

For the analyses of data, Statistical Package for Social Science (SPSS), for Windows version 12, SPSS Inc., Chicago, IL was used. The outliers were visually inspected and confirmed through Normality testing using the Shapiro-Wilk test. Considering the pre-analytical, analytical, and post-analytical errors, all the outliers were removed and data was converted into Gaussian data for further analysis. For the sake of analyses, the studied biomarkers were attributed as independent (Na^+, K^+, and TP) and dependent (LDH, ALT, AST, cTnI, and CK-MB) variables.The RIs were determined through the Reference Value Advisor version 2.1 add-in (freeware v2.1: https://www.biostat.envt.fr/reference-value-advisor/). The overall, age-wise and gender-wise RIs for studied attributes were determined keeping in view the guidelines provided by the American Society of Veterinary Pathology (Friedrichs *et al.*, 2012). The mean (±SE), median, range, and RIs (25th to 95th percentile) were accordingly deduced. The difference between group-wise attributes was tested through an independent *t*-test and ANOVA (*post-hoc* test Duncan's Multiple Range Test) as *per* the number of study groups. A General Linear Model using multivariate analysis was used to find the overall effect of independent serum cardiac attributes on dependent ones.

Ethical approval

The research work was approved by the Departmental Research Ethics Committee, Department of Physiology, IUB, Pakistan, vide Letter No PHYSIO-77/2023-104 dated 13-11-2023.

Results and Discussion

The present study is a preliminary study conducted on apparently healthy Sipli sheep of Pakistan and provides baseline data regarding various cardiac biomarkers and interactions within them. The results will help ascertain any underlying cardiac disorder in sheep in the absence of any clinical signs. The overall results of RIs regarding independent and dependent serum chemistry variables are given in Table 1.

The Na^+ and K^+, either alone or in a ratio (Na^+:K^+), are considered to be the best indicators of cardiac health as confirmed through earlier studies both in humans (Siegel *et al.*, 2008; Ramasamy *et al.*, 2013) and livestock (Yokus and Cakir, 2006). Regarding Na^+, the overall value of the present study (211.8 ± 3.9 mmol/l) and the RI (207.5–264.0 mmol/l) are higher than those reported earlier for different breeds of sheep worldwide. A lower overall value of 153.0 mmol/l and higher RI of 145–160 mmol/l have been reported for big-horn desert-reared sheep (Borjesson *et al.*, 2000). Similarly, a lower overall value of 147.80 mmol/l and lower RIs for Na^+ (143–148 mmol/l) have been reported for the Dorper breed of sheep from South Africa (Santo da Cruz *et al.*, 2017). Merino lambs being reared in Australia under an intensive farming system were studied for various serum chemistry attributes and a lower Na+ value of 147 mmol/l with RI of 138–158 mmol/l has been reported (Lepherd *et al.*, 2009). Even for sheep reared in the USA, a lower value of 148.7 ± 2.5 mmol/l has been reported earlier (Frye *et al.*, 2022). Higher Na^+ and creatinine values in sheep have been considered adaptive physiological phenomena in desert sheep (Norris and Whan, 2008; Soch *et al.*, 2011). Similarly, a comparison of the values of Na^+ attained in the present study with those provided in Merck's Veterinary Manual (Aiello *et al.*, 2016) revealed that the overall values as well as the RIs of the present study are lower than the values given in the manual. It could be a plausible justification that higher than normal values of Na^+ in the Sipli sheep of the present study could either be a physiological adaptation to the hot temperate climate of the Cholistan desert or a difference in the breed.

Considering the overall value (3.3 ± 0.1 mmol/l) and RIs (2.0–6.2 mmol/l) for K^+ in the present study, it was noticed that a higher overall value of 4.7 mmol/l has been reported for bighorn desert-dwelling sheep (Borjesson *et al.*, 2000). However, for these sheep, the RIs (4.0–6.3 mmol/l) are close to the values of the present study. Similar values as of the present study have been reported for Merino adult sheep reared in Australia with RIs of 4.8–5.8 mmol/l (Alonso *et al.*, 1997; Lepherd *et al.*, 2009). On the contrary, a higher value of 5.1 mmol/l has been reported for Merino lambs (Alonso *et al.*, 1997). A report from the USA has also reported a higher K^+ concentration of 5.8 ± 0.5 mmol/l (Frye *et al.*, 2022). Prior studies have elucidated that the timing, method, and technique of blood sampling greatly affect the concentration of K+ in the serum. Furthermore, higher than normal Na^+ and lower than normal K^+ levels in the present study could be suggestive of a physiological adaptive mechanism of Sipli sheep which needs further study.

The overall value for TP in the present study (59.2 ± 1.6 g/l) and its RIs (45.1–95.7) were lower than those reported in previous research reports. The overall value of 66.1 ± 0.26 g/l has been reported for Indigenous Bangaldeshi sheep (Rahman *et al.*, 2018); however, RIs for these sheep were wide (36.0–47.5) as compared to the results of the present study. Similarly, higher values of 73.0 ± 6.1 g/l of TP for sheep of Northeastern USA (Frye *et al.*, 2022) and 70.0 ± 0.7 g/l for Girgentana goats from Italy have been reported (Piccione *et al.*, 2010). Further reports have perceived through their results that the TP tends to decrease in sheep affected with any of the metabolic disorders such as ruminal impaction (Akinrinmade and Akinrinde, 2012). As apparently healthy sheep were selected for the present study hence it seems as a plausible justification that lower than normal values of TP in this study for Sipli sheep may be an inherent characteristic of this breed

Table 1. Overall mean (±SE), median, interquartile range, minimum, maximum, 25th to 95th percentile of RI and 95% confidence interval (CI) for independent and dependent serum chemistry variables in Sipli sheep (n = 141).

Attributes	Mean (±SE)	Median (IQR)	Range (Min-Max)	RI (25th to 95th)	95% CI
Independent attributes					
Na^+(mmol/l)	211.8 ± 3.9	223.0	206.1 (101.9–308.1)	207.5–264.0	204.0–219.7
K^+ (mmol/l)	3.3 ± 0.1	3.2	6.8 (1.1–7.9)	2.0–6.2	3.0–3.6
TP(g/l)	59.2 ± 1.6	58.5	78.3 (21.2–99.5)	45.1–95.7	56.0–62.5
Dependent attributes					
LDH (U/l)	589.5 ± 19	558.5	914.7 (85.0–999.7)	439.1–950.7	551.9–627.0
AST (U/l)	94.0 ± 3.8	82.0	268.0 (22.7–290.7)	60.2–177.3	86.3–101.7
ALT (U/l)	26.1 ± 3.8	19.2	514.2 (3.4–517.6)	14.8–44.1	18.4–33.7
cTnI (ng/ml)	0.1 ± 0.009	0.1	0.8 (0.01–0.9)	0.1–0.3	0.1–0.1
CK-MB (U/l)	155.1 ± 5.3	146.9	280.6 (51.1–331.8)	99.4–264.1	144.4–165.7

ALT = alanine transaminase; AST = aspartate aminotransferase; CK-MB = creatine kinase-myocardial band; cTnI = cardiac troponin I; LDH = lactate dehydrogenase; K^+ = potassium; Na^+ = sodium; TP = total protein.

or a physiological adaptation towards harsh desert climate.

Both the ALT and AST enzymes are considered to be valid and precise biomarkers of hepatocyte necrosis and hepatitis. The AST enzyme is classically lower in patients with hepatocellular injury as compared to ALT in humans. However, this pattern gets the other way around for alcoholic patients (Anderson *et al.*, 2000; van Beek *et al.*, 2013). Regarding sheep, AST has been a better indicator of hepatic health in sheep as compared to ALT being strongly correlated to the age of the animals (Feitosa *et al.*, 2007). In the present study, the overall value of ALT was 26.1 ± 3.8 U/l with RIs of 14.8–44.1. Values of ALT closer to ours have been reported for Girgentana goats from Italy (24.6 ± 1.1 U/l) and for Bangladeshi indigenous sheep (23.4 ± 7.7 U/l) (Piccione *et al.*, 2010; Rahman *et al.*, 2018). Similarly, 23.2 ± 9.6 U/l for serum ALT in Churra-da-Terra-Quente ewes has been reported from Portugal (Dias *et al.*, 2010). Lower values of 15.0 ± 3.0, 14.9 ± 5.3, and 16.8 ± 7.1 U/l have been reported for Arabian goats before pregnancy, during pregnancy, and after pregnancy, respectively (Allaoua *et al.*, 2021). However, these lower values could be denot to the species-oriented difference between sheep and goats. In yet another study, a higher level of ALT has been reported for goats (77.1 ± 4.2 U/l) as compared to healthy sheep (30.4 ± 18.0 U/l) (Kiran *et al.*, 2012). Comparing the RIs for ALT in the present study (14.8–44.1), it was revealed that higher RIs of 60.0–64.2 have been reported for Churra-da-Terra-Quente ewes of Portugal (Dias *et al.*, 2010). However, lower RIs of 15.0–24.0 have been reported for Girgentana goats of Italy (Piccione *et al.*, 2010).

The overall value of AST in the present study was 94.0 ± 3.8 U/l with RIs being 60.2–177.3. A closer overall value of 92.6 ± 2.4 U/l for adult indigenous Brazilian sheep has been reported (De Souza *et al.*, 2019). However, many other studies have reported higher values. A higher value of 137.2 U/l with RIs of 78–312 has been reported for free-ranging desert bighorn sheep (Borjesson *et al.*, 2000) and for Merino sheep being 111.0 ± 2.1 U/l with RIs of 133–147 (Lepherd *et al.*, 2009). This variability in the results of overall values as well as for RIs for AST in Sipli sheep of the present study in comparison to past research work could be ascribed to differences in breed, husbandry practices, and geographical entity (Anderson *et al.*, 2000; Britti *et al.*, 2005; Mostaghni *et al.*, 2005).

One of the main attributes of the present study was determining RIs for cTnI in the Sipli breed of Pakistani sheep for the first time. It has long been determined that cardiac troponins are the first biomarkers to appear in case of any cardiac myopathy, and are the best diagnostic/prognostic biomarkers of myocytic degeneration (Siegel *et al.*, 2008). Extensive work has been conducted on cTnI and cTnT for human medical sciences. Tropinin, in fact, is a protein complex, is a part of the thin filament of heart muscles, and consists of three subunits, i.e., Troponin-I, Troponin-C, and Troponin-T (Azzazy and Christenson, 2002; Wells and Sleeper, 2008; Gunes *et al.*, 2010). Normally, its values are assessed along with those of CK-MB for better diagnostics/prognostics of cardiac myopathy. Though the CK-MB is more specific in cardiac muscles; however, it has low activity in other bodily tissues such as the skeletal, brain, uterus, and brain. Increased levels of both these biomarkers in various livestock and pet animals affected with any of the cardiac pathologies have been reported earlier (Nikvand *et al.*, 2019; Xu *et al.*, 2020; Sepulveda *et al.*, 2022).

The overall values and the RIs for cTnI deduced in the present study for Sipli sheep were 0.1 ± 0.009 ng/ml and 0.1–0.3, respectively. A comparable value of 0.1–

0.3 ng/ml has been reported for Brazilian ewes. This study elaborated that the ewes with pregnancy toxemia had higher values of cTnI of 1.03 ng/ml as compared to healthy ones indicating it is a valid biomarker for pregnancy toxemia (De souza *et al.*, 2019). Yet another study conducted on the ovine model (Italian Sardinian sheep) has reported 0.06 ± 0.003 ng/ml cTnI in healthy adult sheep (Leonardi *et al.*, 2008). In goats, it has been shown that the cTnI is undetectable in healthy goats; however, it takes a gradual increase *per* stage of pregnancy reaching its maximum of 0.494 ng/ml immediately before pregnancy (Tharwat *et al.*, 2012). For studying acute ruminal acidosis in Turkish sheep, a study has reported that the overall value of cTnI for healthy sheep was 0.035 ± 0.015 ng/ml (Kirbas *et al.*, 2014) which is lower than the value of the present study. However, another study from Turkey has reported an overall value of 0.02 ± 0.001 ng/ml for cTnI in ewes (Tumer *et al.*, 2021). The comparison of our results for cTnI with prior studies indicates that there is a nominal level of cTnI in sheep which has a wider range and RI. In the present study, the overall value for CK-MB was 155.1 ± 5.3U/l with the RIs of 99.4–264.1. Closer values of 168.2 U/l have been reported for Iranian sheep (Ataollahi *et al.*, 2013). Similar values as that of our study (151.69 ± 45.0 U/l) have been reported for Turkish ewes (Tumer *et al.*, 2021). A lower value of 50.7 ± 7.2 U/l has, however, been reported for Brazilian Indigenous sheep (De souza *et al.*, 2019). The RIs of the present study are, however, within the range provided by Merck's Veterinary Manual (Aiello *et al.*, 2016). Comparison with prior studies indicates that the values of CK-MB of the present study are generally in line with those published for sheep earlier though the RIs differ and have a wide range for sheep. The slight differences could be credited to differing husbandry practices and breed variation.

Taking the results of the present study of LDH in perspective, an overall value of (589.5 ± 19 U/l) and RIs of 439.1–950.7 were noticed. A comparable value of 534.0 U/l with RIs of 409–788 has earlier been reported for Bighorn desert sheep (Borjesson *et al.*, 2000). A closer value of 583.8 ± 83.6 U/l with RI of 479–705 has been reported for sheep from Southern Punjab, Pakistan (Kiran *et al.*, 2012). This study also reported a lower LDH value of 304.2 ± 143.0 U/l for goats in Southern Punjab which may be a species-oriented difference. A lower RI of 238–440 for LDH in sheep has been given in Merck's Veterinary Manual (Aiello *et al.*, 2016). An even lower value of 120.9 ± 4.4 U/l has been reported for sheep in Greece (Katsoulos *et al.*, 2010). This difference and a wide range of RIs presented in prior work as well as of the present study indicate a wide variation in this attribute as per geo-location, feeding regimen, and method of determining LDH (Katsoulos *et al.*, 2010; Sepulveda *et al.*, 2022).

Gender-wise results of RIs regarding dependent and independent serum chemistry variables of the study are given in Table 2. The Na^+ was statistically ($p \leq 0.05$) higher for females (225.9 ± 2.8 mmol/l) as compared to that in males (142.1 ± 9.7 mmol/l). However, K^+ and TP were non-significant ($p \geq 0.05$) between males and females. While assessing serum biochemical reference values for indigenous sheep of Bangladesh, similar results have been reported for TP in females (6.9 ± 0 g/dl) and males (6.2 ± 0.5g/dl), not being different (Rahman *et al.*, 2018). Results compared to those of Girgentana goats of Italy also have noticed no difference for K^+ and TP between male and female goats though Na^+ was higher in females as compared to male goats which is in line with the results of the present study (Piccione *et al.*, 2010). Extensive studies conducted on the gender-based differences in the hematochemical profile of sheep have globally endorsed that female sheep have a higher demand and hence intake of salt, especially during pregnancy, which leads to higher levels of serum concentration of Na^+ (Soch *et al.*, 2011; Kiran *et al.*, 2012; Badawi and AL-Hadithy, 2014,). Further studies on serum chemistry attributes of sheep during different stages of pregnancy could provide elaborate information on this phenomenon.

Similarly, regarding dependent variables of the study, it was noticed that females had statistically higher ($p \leq 0.05$) values for LDH (591.7 ± 19.5 U/l), AST (95.5 ± 4.1 U/l) and CK-MB (160.7 ± 6.3 U/l) as compared to that in males. However, ALT and cTnI were not different between the gender groups. Comparing these results with prior reports, it was revealed that variable results have been reported regarding gender-based differences in these biochemical parameters with certain researchers reporting a significant difference while others not. For Indigenous sheep of Bangladesh, the values of ALT for male and female sheep have been reported as 23.3 ± 7.3 and 23.4 ± 8.2 U/l, respectively being similar for both genders (Rahman *et al.*, 2018). However, a similar study has reported a higher value of AST for males (102.7 ± 26.8 U/l) as compared to that in female sheep (96.4 ± 25.7 U/l). On the contrary, a study conducted on Arabian goats has reported that age, gender, and stages of pregnancy had no effect on ALT and AST values (Allaoua *et al.*, 2021).

Age-wise results of studied attributes for apparently healthy Sipli sheep are given in Table 3. The Na^+ was significantly higher in the present study for G1 animals (up to 1 year) (218.9 ± 5.8 mmol/l) followed by that in G2 animals (from 1 to 2 years) (213.2 ± 5.3 mmol/l) and G3 animals (above 2 years) (198.1 ± 11.6 mmol/l). However, K^+ and TP were not significantly different for the three study groups. A report on Girgentana goats of Italy has reported that young animals (up to 2 years) had lower Na^+ (135.8 ± 0.8 mmol/l) as compared to adult ones (above 2 years) (141.3 ± 1.0 mmol/l) (Piccione *et al.*, 2010). Our results are not in line with those reported for South African Dorper sheep (Santo da Cruz *et al.*, 2017) and in Merino sheep (Lepherd *et al.*, 2009) in which Na^+ and Cl^- were not affected either by age

Table 2. Overall mean (±SE), median, minimum, maximum, range, 25th to 95th percentile of RI and 95% CI for dependent and independent serum chemistry attributes as affected by gender in apparently healthy Sipli sheep (n = 141).

Groups	Mean ± SE	Median	(Min-Max)	Range	RI (25th to 95th)	95% CI
Independent attributes						
Sodium (mmol/l)						
Females (n = 109)	225.9 ± 2.8*	226.5	101.9–308.1	206.1	217.5–270.9	220.2–231.6
Males (n = 22)	142.1 ± 9.7	123.0	108.1–259.7	151.6	117.5–254.8	121.9–162.3
Potassium (mmol/l)						
Females (n = 109)	3.3 ± 0.1	2.8	1.1–7.9	6.8	1.8–6.4	2.9–3.6
Males (n = 19)	3.7 ± 0.1	3.7	2.5–4.8	2.3	3.3–4.7	3.4–4.0
TP (g/l)						
Females (n = 99)	59.3 ± 1.8	58.2	21.2–99.5	78.3	44.7–95.8	55.6–63.1
Males (n = 29)	58.7 ± 3.2	58.9	25.0–95.7	70.6	45.7–93.1	52.1–65.4
Dependent attributes						
LDH (U/l)						
Females(n = 109)	591.7 ± 19.5*	570.7	85–999.7	914.7	441.1–947.1	553.0–630.4
Males (n = 05)	554.5 ± 83.8	481.6	319.7–999.7	679.9	429.0-667.8	349.2–759.7
AST (U/l)						
Females(n = 109)	95.5 ± 4.1*	83.8	22.7–255.7	233.0	61.1–175.0	87.3–103.8
Males (n = 20)	88.0 ± 9.9	66.3	37.5–290.7	253.1	57.8-243.5	67.6–108.4
ALT (U/l)						
Females(n = 109)	22.8 ± 1.1	19.2	3.4–93.4	89.9	15.7–43.6	20.5–25.1
Males (n = 15)	40.9 ± 20.8	17.4	8.7–517.6	508.9	13.3-405.5	−2.2–84.1
cTnI (ng/ml)						
Females(n = 109)	0.1 ± 0.01	0.1	0.01–0.9	0.8	0.1–0.3	0.1–0.1
Males (n = 32)	0.1 ± 0.01	0.1	0.01–0.5	0.4	0.1–0.3	0.1–0.1
CK-MB (U/l)						
Females (n = 49)	160.7 ± 6.3*	161.7	51.1–331.8	280.6	101.5–265.8	148.0–173.3
Males (n = 31)	132.4 ± 7.0	132.0	82.5–232.7	150.2	95.7–210.9	117.9–146.9

*Significant at $p \leq 0.05$ between males and females.

or gender. The varying results are endorsed by the fact that Na^+ and many other biochemical electrolytes (K^+, Cl^-) are majorly affected by the status of hydration of the animal and intra-individual differences (Borjesson *et al.*, 2000, Britti *et al.*, 2005; Badawi and AL-Hadithy, 2014). The results of the present study for TP are in line with those reported for three indigenous sheep breeds of Kurdistan in which it was reported that breed, age, and gender had no effect on K^+ and TP levels (Oramari *et al.*, 2014). Gradual increase in presently studied age groups may be a genetic predilection of the Sipli sheep breed or due to variation in climatic conditions.

In the present study, CK-MB was higher in G3 animals (166.0 ± 10.6 U/l). Prior research work conducted on humans has established that there is an age-related increase in CK-MB (Perryman *et al.*, 1984; Malasky and Alpert, 2002). Furthermore, CK-MB seems to be elevated without any clear cardiac myopathy as its release is episodic and enhances with skeletal muscle activity in humans (Malasky and Alpert, 2002). For comparison purposes, there is no study conducted on the age-related trend of CK-MB in small ruminants. The same applies to cTnI, which showed no difference within the three age groups of Sipli sheep in the present study. Regarding dependent variables, LDH and CK-MB were statistically different ($p \leq 0.05$) between the three age groups. The LDH was significantly higher ($p \leq 0.05$) in G1 animals (618.8 ± 34.3 U/l) whereas CK-MB was higher in G3 animals (166.0 ± 10.6 U/l). The AST, ALT, and cTnI were non-significantly different ($p \geq 0.05$) between the three studied age groups.

According to the results of multivariate analyses, (Table 4), Na^+ had a statistically higher ($p \leq 0.01$) positive correlation with LDH (r = 0.233) whereas K^+

Table 3. Overall mean (±SE), median, minimum, maximum, range, 25th to 95th percentile of RI) and 95%CI for dependent and independent serum chemistry attributes as affected by age in apparently healthy Sipli sheep (n = 141).

Groups	Mean ± SE	Median	(Min-Max)	Range	RI (25th to 95th)	95% CI
Independent variables						
Sodium (mmol/l)						
G1 (n = 37)	218.9 ± 5.8[a]	222.1	121.1–291.3	170.2	218.9 ± 5.8	222.1
G2 (n = 62)	213.2 ± 5.3[b]	225.4	101.9–308.1	206.1	213.2 ± 5.3	225.4
G3 (n = 26)	198.1 ± 11.6[c]	218.5	108.1–288.8	180.6	198.1 ± 11.6	218.5
Potassium (mmol/l)						
G1 (n = 37)	3.4 ± 0.2[a]	3.1	1.1–7.7	6.6	1.8–7.2	2.8–4.0
G2 (n = 68)	3.3 ± 0.1[a]	3.2	1.1–5.7	5.7	2.1–5.9	2.9–3.6
G3 (n = 23)	3.3 ± 0.3[a]	3.64	1.4–7.9	6.4	1.8–1.8	2.6–3.9
TP (g/l)						
G1 (n = 35)	58.1 ± 3.1[a]	59.1	21.2–97.7	76.5	44.6-96.5	51.6–64.6
G2 (n = 63)	58.9 ± 2.4[a]	57.8	22.3–99.5	77.2	43.1-95.7	54.0–63.8
G3 (n = 30)	61.0 ± 2.9[a]	58.5	34.4–95.8	61.3	45.8-91.7	54.9–67.1
Dependent variables						
LDH (U/l)						
G1 (n = 37)	618.8 ± 34.3[a]	607.1	186.1–999.7	813.5	453.3–999.7	549.0–688.5
G2 (n = 68)	564.2 ± 24.4[b]	512.0	85.0–999.7	914.7	420.9–960.4	515.4–612.9
G3 (n = 11)	647.2 ± 59.5[c]	594.9	416.8–947.1	530.2	477.6–938.2	514.5–779.8
AST (U/l)						
G1 (n = 37)	92.3-7.0[a]	76.8	45.4–199.9	154.5	61.5–178.7	78.0–106.5
G2 (n = 62)	92.3 ± 5.3[a]	81.6	22.7–255.7	233.0	60.2–201.7	81.7–103.0
G3 (n = 32)	99.4 ± 9.2[a]	92.5	40.1–290.7	250.5	58.7–214.6	80.6–118.3
ALT (U/l)						
G1 (n = 37)	22.6 ± 2.0[a]	19.2	3.4–66.3	62.8	14.8–48.2	18.5–26.7
G2 (n = 68)	28.8 ± 7.3[a]	18.7	9.6–517.6	508.0	14.8–42.8	14.0–43.6
G3 (n = 28)	23.9 ± 2.9[a]	20.0	8.7–93.4	84.6	17.6-71.8	17.9–30.0
cTnI (ng/ml)						
G1 (n = 37)	0.1 ± 0.02[a]	0.1	0.01–0.7	0.6	0.1–0.4	0.09–0.1
G2 (n = 68)	0.1 ± 0.008[a]	0.1	0.01–0.5	0.4	0.1–0.2	0.09–0.1
G3 (n = 36)	0.1 ± 0.02[a]	0.1	0.01–0.9	0.8	0.1–0.3	0.09–0.1
CK-MB (U/l)						
G1 (n = 37)	159.4 ± 9.6[a]	148.5	67.6–257.5	189.8	103.1–257.5	139.8–179.0
G2 (n = 68)	147.7 ± 8.0[b]	137.0	51.1–331.8	280.6	94.0–274.6	131.7–163.7
G3 (n = 29)	166.0-10.6[c]	168.4	84.1–287.2	203.0	113.9–275.3	144.2–187.8

age groups (G1 = up till 1 year, G2 = from 1 to 2 years, G3 = above 2 years).

[a,b,c], different superscripts between the groups are different at $p \leq 0.05$.

had a statistically higher ($p \leq 0.01$) negative correlation with LDH (r = −0.587), AST (r = −0.456) and CK-MB (r = −0.495). The Na^+ significantly ($p \leq 0.05$) predicted AST (adj. r-square = 0.976, 97% probability) and CK-MB (adj. r-square = 0.477, 47% probability). Similarly, K^+ significantly ($p \leq 0.05$) predicted LDH (adj. r-square = 0.626, 62% probability) and ALT (adj. r-square = 0.897, 89% probability). The TP had the highest adjusted r-square of 0.770 (77% probability) with AST; however, it was statistically non-significant ($p \geq 0.05$).

Table 4. Between subject effects deduced through general linear model using multivariate analysis between independent and dependent serum attributes.

Attributes	*r*- values	*F* value	Sig.	Adj. *r*-square
Sodium (mmol/l)				
LDH (U/l)	0.233**	1.441	0.225	0.279
AST (U/l)	0.110	48.065	0.000*	0.976*
ALT (U/l)	−0.130	1.109	0.441	0.087
cTnI (ng/ml)	−0.126	0.760	0.816	−0.255
CK-MB (U/l)	0.109	2.07	0.031*	0.477*
Potassium (mmol/l)				
LDH (U/l)	−0.587**	2.942	0.009*	0.626*
AST (U/l)	−0.456**	0.877	0.670	−0.118
ALT (U/l)	−0.57	11.33	0.000*	0.897*
cTnI (ng/ml)	−0.078	0.482	0.991	−0.775
CK-MB (U/l)	−0.495**	1.513	0.145	0.302
TP (g/l)				
LDH (U/l)	0.015	0.306	0.958	−0.14
AAT (U/l)	0.043	1.515	0.481	0.770
ALT (U/l)	0.037	4.4	0.203	0.336
cTnI (ng/ml)	0.064	2.228	0.361	0.547
CK-MB (U/l)	0.063	1.319	0.529	0.239

**signifcant at $p \leq 0.01$; *significant at $p \leq 0.05$.

Conclusion

In a nutshell, the RIs for cardiac serum biomarkers included in the present study for Sipli sheep are generally within the reference range provided for sheep with slight variations and can be used as diagnostic/prognostic biomarkers for health assessment in sheep breeds being reared in tropical/subtropical areas of the world. The Na^+ levels of Sipli sheep serum were higher than the normal range for sheep and may be attributed to physiological adaptation to harsh desert environments or breed characteristics. A nominal level of cTnI in Sipli sheep had a wider range and RI, and alone did not provide reliable prediction of cardiac health in sheep. The Na^+ and K^+ strongly predicted AST, CK-MB, LDH, and ALT which can be used for cardiac assessment of sheep. The results of the study present baseline data about these cardiac biomarkers which may be utilized for cardiac assessment of the sheep. It is recommended that the serum cardiac biomarkers ascertained in this study may be studied in perspective to electrophysiology and ultrasonography of sheep heart for confirmed diagnosis/prognosis of cardiomyopathies. Furthermore, while assessing the health status of livestock, the RIs of local animals deduced under local climatic conditions may be used as a yardstick instead of comparing with RIs published in other geographic entities of the world. Future studies may incorporate animals with cardiac pathologies for comparative purposes.

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Conflict of interest

The authors declare no competing interests.

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Authors' contributions

Umer Farooq and Mushtaq Husaain Lashari: Conceptualization and design. Musadiq Idris and Madiha Sharif: Lab analyses and data collection. Aisha Mahmood: Data analyses. Musarrat Abbas Khan: Writing, editing, and reviewing the manuscript.

Data availability

All the relevant data are within the manuscript and no additional data source is needed.

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