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Hematological and Biochemical Analysis of Blood of Fresh Water Turtles (Order: Testudines) from River Indus (Guddu), Sindh, Pakistan

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ABSTRACT

The main aim of current research study is to compare hematological and biochemical parameters of seven freshwater turtle species (Order Testudine). For this purpose, a total of 30 blood samples were collected from different turtle species including *Aspideretes (A) gangeticus*, *A. hurum*, *Chitra indica*, *Lissemys punctata*, *Kachuga (K) tecta*, *K. smithi* and *Hardella thurjii* exhibiting different habitats in the Guddu Barrage, Indus River, Pakistan. Hematological and biochemical analysis showed a significantly higher and lower values of red blood cells in *Pangshura tecta* and *Kachuga smithii*, PDW in *Nilssonia hurum* and *Kachuga smithii*, MCV in *Chitra indica* and *Kachuga smithii*, haemoglobin in *Chitra indica* and *Kachuga smithii*, PLT in *Pangshura tecta* and *Chitra indica*, MPV in *Nilssonia hurum* and *Lissemys punctata*, TLC in *Nilssonia hurum* and *Kachuga smithii*, LYM in *Chitra indica* and *Kachuga smithii*, NEU in *Chitra indica* and *Kachuga smithii*, EOS in *Hardella thurjii*, *Lissemys punctata* and *A.gangeticus*. Mean values of BAS were examined zero in all freshwater turtle species, MO in *Lissemys punctata*, *Hardella thurjii* and *Pangshura tecta*, and PCV in *Nilssonia hurum* and *Kachuga smithii*, respectively. Additionally, a significantly highest and lowest serum levels of glucose were examined in *Kachuga smithii* and *Pangshura tecta*, protein in the species of *Pangshura tecta* and *kachuga smithii*, cholesterol in *Nilssonia hurum* and *Chitra indica*, urea in *A. gangeticus* and *Chitra Indica*, triglycerides in *Nilssonia hurum* and *Chitra indica* and uric acid in *Pangshura tecta* and *Lissemys punctata*, respectively. Finally, no significant ($p< 0.5$) differences were identified in the hematological and biochemical parameters of seven species except EOS and MO. Based on these findings, we propose blood profiling as a nutritional tool to monitor health and disease in both wild and freshwater turtles.

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Authors' Contribution
KH collected the data during the field work and did research experiment in the laboratory. MSC helped in writing the paper. GSG helped to provide the basic ideas of research design to complete this research study.

Key words
Hematological, Biochemical parameters, Freshwater turtle,

INTRODUCTION

Turtles are testudines diapsids or chelonii and are considered distinct because their ribs perform functions as a separate bony or cartilage shell (Hutchinson, 1996; Dubois and Roger, 2010). The earliest known members of this group date back to 220 million years and are referred as fresh water and marine testudines (Robert *et al*., 2008). According to the Asian Working Group on Turtle Trade as well as Species Survival Commission of Tortoise and Freshwater Turtle of the International Union for Conservation of Nature, more than half of Asian freshwater tortoise are considered endangered. Among these, a total of 18 are ranked as critically endangered species (Kurta *et al*., 2007). There are at least 167 reptile species (Ghalib and Hasnain, 2017) and at least 8 species of Pakistan's freshwater turtles are recorded in Pakistan. Amongst freshwater turtles, soft-shell turtles include *Aspideretes gangeticus, Aspideretes hurum, Chitra indica* and *Lissemys punctata*. On the other hands, species of hard-shell include *Geoclemys hamiltonii, Kachugu tecta, Kachugu smithii* and *Hardella thurjii*.

Due to continued diminishing of turtle population, the aquatic ecosystem is collapsing particularly in areas which are under-studied and suffer from biodiversity losses (Noureen and Khan, 2007). In Pakistan, the trade of freshwater turtles was first demonstrated in the 1990s (Noreen, 1996). The Guddu Barrage on the Indus River carry three channel system which is considered essential for biodiversity of aquatic life. This represents world's largest station which is made up of two channels on the right bank. The river Indus is a large river which flows from Tibet into India and Pakistan. The Basin in the River Indus is very

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productive area and is enriched with freshwater fauna (freshwater turtles). The biochemical and hematological plasma variables are essential to the management of endangered fresh-water turtles including Indus mud turtles (*Lissemys punctata*) and other fresh-water turtle species. Blood parameters are important in the estimation of physiological disorders of chelonians and to provide valuable diagnostic and prognostic markers for diseases (Oleivera-Junior *et al*., 2009). However, studies of blood biochemistry and hematology are limited and variable criteria for certain concentrations have been proposed (Casal *et al*., 2009). Blood samples for hematological and biochemical examination have been obtained from the dorsal coccygeal vein (Yilmaz and Tosunolu, 2010) for analysis. However, many factors including environmental conditions, age and sex, may influence the values of chelonian biochemistry. Relative to males, females often have higher values of albumin, calcium, cholesterol, phosphorus and triglycerides that are usually attributed to vitellogenesis (Gregory *et al*., 2014).

Reptilian hematological reptile research can be traced back to 1940s when a substantial increase in literature related to microstructure and ultrastructure of chelonian blood cells has been recorded. However, few studies have been conducted on haematopoiesis, cytochemical characterization, and blood cell growth. For the evaluation and regulation of chelonian health status, normal hematologically and biochemically reference areas have been considered (Christopher, 2007; Figueroa, 2005; Zhang *et al*., 2011). Specifically for clinically endangered species, gathering such information will be critical for planning of management strategies (Bolten and Bjorndal, 1992). Hematological tests have also provided important data for monitoring rehabilitated chelonists before they are released in the wild (Casal *et al*., 2009; Zhang *et al*., 2011).

Packed cell volume (PCV) and eosinophils count show trends in gender differences, ratio of heterophils and monocytes, total protein, uric acid, cholesterol and triglycerides (Dias *et al*., 2009). Compared to poor and usual nutritional conditions of giant amazon and malnourished turtles, the number of red blood cells reduced significantly without affecting homeostatsis. However, severe normocytic-hypocromic anaemia and significant immune depression, hematocrites, plasma glucose, complete plasma protein, cholesterol and urea can be underlining causes. The lower hematocrit values instigate the standards for malnourished turtles (Frair, 1977). It was argued by (Christopher, 1999) that in the wild desert tortoise (*Gopherus agassizii*), the reduced level of hematocrit value was caused due to the chronic malnutrition in the animals which were captured without intake of food for the period of 11 months.

Various studies have been conducted to define the characteristics of the general profile of the blood (Dessauer, 1970; DuGuy, 1967; Frye, 1991; Campbell, 1996; Stein, 1996). The blood parameters of reptiles that are influenced by different factors such as the seasonal variation, reproduction, animal age and sex (Dessauer, 1970; Frye, 1991; Wilkinson, 2003) and these blood parameters may be altered by the annual cycle or even throughout the life cycle of animals.

It is also evident that in few species of turtles, physiological disturbances indicate diseases in animals (Swimmer, 2000; Christopher *et al*., 2003; Knotkova *et al*., 2005; Olivera-Junior *et al*., 2009), trauma (Knotkova *et al*., 2005) or expositions to pollutants (Lutcavage *et al*., 1995; Keller *et al*., 2004). Additionally, frequent anaemia and hypoproteinemia consequently predict the animal health and thus propose a suitable therapeutic target Campbell, 1996; Nortn, 2000).

In the laboratory cultured environments, the starved species of *Chilonia mydas,* the biochemical parameters for glucose, and plasma non-protein nitrogen levels generally led to the creation of urea (Bonnet, 1979; Perrault *et al*., 2001; Work *et al*., 1998; Zhang *et al*., 2011). The glucose level did not alter in *Phrynopus hilarii* despite decreased metabolic production and malnourished was witnessed (Campbell, 1996; Silva and Migliorini, 1990). However, it is difficult to setup approaches of conservation and management due to the scarcity of reliable information or reptilian species in Pakistan.

The major aim of the current research is to examine biochemical and hematological blood parameters in seven species of freshwater turtle from the territory of Indus River, Guddu Barrage, Sindh, Pakistan.

MATERIALS AND METHODS

Study area

The study was carried out in the Guddu River channel and around the Indus River wetlands adjacent to the Guddu barrage, Sindh Province, Pakistan. Guddu Barrage is an important barrage on the Indus River near the Kashmore region of Pakistan's Sindh Province. The Guddu barrage has a large three-channel structure. Two channels on the right bank are among the world's largest channels. Coordinates between latitude vary from 28°25' to 59.99 North and longitude range from 69°43 to 59.99 East. It feeds the feeder for Ghotki, the feeder for Begari, the feeder for the desert and the canals for pat feeder.

Collection of turtles

In the present study, we captured seven different

species of freshwater turtles (out of eight species) from Guddu River Sindh during the month of May 2018 to September 2019. We collected four different softshell species and three freshwater turtle hard-shell species from Indus River, Guddu. The species we found are as under: *Lissemys punctata, Aspideretes gangeticus*, *Chitra indica*, *Kachuga smithi, Aspideretes hurum, Kachuga tecta*, and *Hardella thurjii.*

Collection of blood sampling

A total of 30 samples from each wild freshwater turtle species were collected. The blood samples were taken using coccygeal vein, femoral vein and jugular vein,using 22-gauge needles and 3.0 ml sodium heparin-coated syringe. Two samples were collected from each turtle: first an aliquot of 2 ml of whole blood in heparinized vacutainers and the other 2 ml of blood in the serum chemistry gel vacutainer. For the leukocyte differential count and determination of packed cell volume (PCV) fresh blood was used. For RBC and WBC, heparinized blood was used. An automatic biochemical analyzer (Dias *et al*., 2009) was used for hematology analysis to calculate plasma biochemical concentrations using an automated hematology analyzer. Immediately after collection, samples were placed in the ice box and brought to the laboratory where analysis was performed. The biochemistry of plasma glucose (mg/dl), protein (mg/dl), cholesterol (mg/dl), urea (mg/dl), triglycerides (mg/dl) and uric acid (mg/dl) were analysed by an automatic biochemistry analyzer and centrifuged for 10 min at 3000 rpm and the section of plasma was isolated from the tortoise blood cells (Yilmaz and Tosunolu, 2010). Analysis of hematological parameters of red blood cell (RBC 210 mm^3), platelet distribution width (PDW), mean cell volume (MCV), haemoglobin (HB), platelet (PLT), mean platelet volume (MPV), total leukocyte count (TLC), lymphocytes (LYM), neutrophils (NEU), eosinophils (EOS), monocytes (MO), basophils (BAS) was performed by automatic analyzer and blood samples were loaded into the analyzer within 10 min of collection of samples.

Statistical analysis

The data collected were tabulated and statistically analysed. Student *t-test* was applied to compare freshwater turtles hematological and biochemical attributes using social science statistical package (SPSS-21). Mean ± SD; SEM data were reported and significant differences were considered at $p<0.05$.

RESULTS AND DISCUSSION

In the present study, hematological and biochemical values were calculated to generate reference values in the freshwater turtles (Order Testudines) collected from River Indus Guddu region of the Sindh Province of Pakistan. During the months of May 2018 to September 2019, a total of 30 samples were obtained from freshwater turtle species. In the months of May 2018 to November 2018, four different species were collected, and three different freshwater turtles were collected between April and September 2019. Four of them were identified to be hard-shell turtles and four other species were reported as softshell turtles. We demonstrated the hematology and serum biochemistry parameters of *Aspideretes gangeticus, Aspideretes hurum, Chitra indica, Lissemys punctata, Kachuga tecta, Kachuga smithii* and *Hardella thurjii*.

The hematological and blood biochemical values collected from the wild environment showed a significant fluctuations with respect to each other (Table I).

The highest and lowest mean values of RBC were examined (0.56±0.65 and 0.32±0.16) in the species of *Pangshura tecta* and *Kachuga smithii*, respectively (Table I). The values of RBC showed that there was no significance difference among all species (Table I). The highest and lowest mean values of PDW were examined in the species of *Nilssonia hurum* and *Kachuga smithii*, and found to be 27.75±10.53 and 24.17±13.86, respectively (Table I). The values of PDW showed that there was no significance difference between all species (Table I). The highest and lowest mean values of MCV were examined in the species of *Chitra indica* and *Kachuga smithii*, and were identied as 90.70±27.31 and 75.40±38.13, respectively (Table I). The values of MCV showed that there was no significance difference ($P< 0.05$) among all the species (Table I). The highest and lowest mean values of HB were dentified (6.34±2.03; 5.68±2.16) in the species of *Chitra indica* and *Kachuga smithii* (Table I). The HB values analysis revealed that there was no significance difference among all the species (Table I). The highest and lowest mean values of PLT were examined as 27.20±6.95 and 25.20±7.78 in the freshwater species of *Pangshura tecta* and *Chitra indica*, respectively (Table I). The values of PLT showed that there was no significance difference ($P< 0.05$) among all species (Table I). The highest and lowest mean values of MPV were examined (6.23±1.76 and 6.02±1.73) in the freshwater species of turtles *Nilssonia hurum* and *Lissemys punctata*, respectively (Table I). The analysis of MPV values showed that there was no significance difference ($P< 0.05$) among all species (Table I). The highest and lowest mean values of TLC were recorded as 71.53±22.27 and 64.52±21.52 in the species of *Nilssonia hurum* and *Kachuga smithii*, respectively (Table I). The values for TLC showed that there was no significance difference ($P< 0.05$) among all the examined

species. The highest and lowest mean values of LYM were examined 60.96±17.16 and 54.76±25.57 in the species of *Chitra indica* and *Kachuga smithii*, respectively (Table I). The values of LYM showed that there was no significance difference (P< 0.05) among all species (Table I). The highest and lowest mean values of NEU were examined and found to be 64.96±56.49 and 60.00±56.18 in the species of *Kachuga smithii* and *Hardella thurjii*, respectively (Table I). The values of NEU showed that there was no significance difference (P< 0.05) among examined species (Table I). The highest and lowest mean values of EOS were examined in the species of *Hardelaa thurjii* and *Lissemys punctata; A. gangeticus* and found to be 1.90±0.30 and 1.00±0.00, respectively (Table I).

Analysis indicated that *Lissemys punctata* differed from *K. smithii*, *A. hurum* and *H. thurjii* while others remained similar or showed no significance difference (P< 0.05) compared to other species (Table I). The parametric analysis showed that the *A. ganeticus* was different from *K. smithii*, *A. hurum* and *H. thurjii* while others remained similar or there was no significance difference (P< 0.05) between them (Table I). The values showed that *K. smithii* was different from LP, *A. gangetics*, *Chitra indica* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The values showed that the *A. hurum* is differed from LP, *A. gangeticus*, *Chitra indica*, *K. tecta* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The analysis of values showed that *Chitra indica* was different from *A. hurum* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The values showed that *K. tecta* was differ from *A. hurum* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The values showed that *H. thurjii* was different from all other groups. The mean values of BAS were examined zero in all the studied freshwater turtle species (Table I).

The highest and lowest mean values of MO were identified to be 2.00±0.00 and 1.40±0.49 in *Lissemys punctata*; *Hardella thurjii* and *Pangshura tecta*, respectively (Table I). The values showed that the *Lissemys punctata* was different from *A. gangeticus*, *Chitra indica*, and *K. tecta* while others were similar or there ws no significance difference (P< 0.05) between them (Table I). The significance values showed that the *A. gangeticus* was different from LP and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The significance values showed that *K. smithii* was different from *K. tecta* while others were identical or there was no significance difference (P< 0.05) between them (Table I). The values showed that *A. hurum* was different from *Chitra indica* and *K. tecta* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The values showed that the *Chitra indica* is differ from LP, *A. hurum* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The significance values showed that *K. tecta* was different from LP, *K. smithii*, *A. hurum* and *H. thurjii* while others were similar or there was no significance difference (P< 0.05) between them (Table I). The analysis of values showed that *H. thurjii* was different from *A. gangeticus*, *Chitra indica* and *K. tecta* while others were similar or there was no significance difference (P< 0.05) between them (Table I).

The highest and lowest mean values of PCV were examined to be 4.36±4.32 and 2.73±1.06 in the species of *Nilssonia hurum* and *Kachuga smithii*, respectively (Table I). The analysis of values of PCV showed that there was no significant difference (P< 0.05) among all the species (Table I). The highest and lowest mean values of glucose were examined in the species of *Kachuga smithii* and *Pangshura tecta* and found to be 1.25±14.38 and 1.19±16.39, respectively (Table I). The significance values of glucose showed that there was no significant difference (P< 0.05) among all species (Table I). The highest and lowest mean values of protein were examined as 3.22±1.07 and 2.82±0.88 in the species of *Pangshura tecta* and *kachuga smithii*, respectively (Table I). The values of protein showed that there was no significant difference (P< 0.05) among all species (Table I). The highest and lowest mean values of cholesterol were examined in the species of *Nilssonia hurum* and *Chitra indica* and found to be 1.56±54.43 and 1.38±15.28, respectively (Table I). The values for cholesterol showed that there was no significant difference (P< 0.05) among all species (Table I). The highest and lowest mean values of urea were examined in the species of *A. gangeticus* and *Chitra indica*, and found to be 23.13±3.21 and 22.36±2.67, respectively (Table I). The values of urea showed that there was no significant difference (P< 0.05) among all the species (Table I). The highest and lowest values of triglyceride were examined to be 94.00±20.77; 84.90±11.67 in the species of *Nilssonia hurum* and *Chitra indica*, respectively (Table I). The values of triglyceride showed that there was no significant difference (P< 0.05) among all the species (Table I). The highest and lowest mean values of uric acid were examined as 2.01±0.76 and 1.83±0.38 in the species of *Pangshura tecta* and *Lissemys punctata*, respectively (Table I). The analysis of the uric acid quantitied showed that there was no significant difference (P< 0.05) among examined species (Table I).

Table I. Comparison analysis of hematological and biochemical parameters of seven species of freshwater turtles. Different letters in the same line indicate significant difference (P≤0.05). The values are Mean±SD.

Species	*Lissemys punctata*	*A.gangeticus*	*Kachuga smithii*	*Nilssonia hurum*	*Chitra indica*	*Pangshura tecta*	*Hardella thurjii*	F value	Sig
RBC (10 mm^3)	0.34±0.14^a	0.34±0.14^a	0.32±0.16^a	0.54±0.61^a	0.36±0.11^a	0.56±0.65^a	0.42±0.29^a	2.152	.049
PDW (%)	24.53±13.91^a	25.88±12.93^a	24.17±13.86^a	27.75±10.53^a	25.87±12.35^a	25.20±12.58^a	25.40±12.62^a	.251	.958
MCV (%)	81.00±32.20^a	86.43±24.03^a	75.40±38.13^a	88.66±25.09^a	90.70±27.31^a	88.46±23.27^a	84.24±23.21^a	1.069	.382
HB (%)	5.86±2.04^a	6.10±2.00^a	5.68±2.16^a	6.32±1.98^a	6.34±2.03^a	6.00±2.09^a	5.76±1.90^a	.494	.812
PLT (%)	25.90±8.17^a	26.53±7.74^a	25.23±8.39^a	26.73±8.16^a	25.20±7.78^a	27.20±6.95^a	25.71±8.24^a	.281	.945
MPV (%)	6.02±1.73^a	6.12±1.58^a	6.04±1.75^a	6.23±1.76^a	6.12±1.58^a	6.17±1.74^a	6.16±1.57^a	.056	.999
TLC (%)	66.33±20.26^a	70.50±23.47^a	64.52±21.52^a	71.53±22.27^a	70.32±18.98^a	66.77±19.87^a	70.35±17.49^a	.510	.801
LYM (%)	57.30±22.23^a	58.76±20.46^a	54.76±25.57^a	58.10±20.57^a	60.96±17.16^a	56.46±22.75^a	60.70±18.67^a	.331	.920
NEU (%)	61.46±56.67^a	62.10±56.14^a	64.96±56.49^a	62.26±56.22^a	60.10±57.30^a	62.20±56.59^a	60.00±56.18^a	.026	1.00
EOS (%)	1.00±0.00^a	1.00±0.00^a	1.36±0.49^{b,c}	1.40±0.49^c	1.03±0.18^a	1.13±0.34^{a,b}	1.90±0.30^d	30.64	.000
BAS (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00
MO (%)	2.00±0.00^c	1.63±0.49^{a,b}	1.80±0.40^{b,c}	1.83±0.37^{b,c}	1.46±0.50^a	1.40±0.49^a	2.00±0.00^c	11.57	.000
PCV (%)	2.91±0.77^a	3.06±.62^a	2.73±1.06^a	4.36±4.32^a	3.27±0.90^a	3.57±3.61^a	3.07±.61^a	1.768	.107
Glucose (mg/dL)	1.23±14.25^a	1.22±15.86^a	1.25±14.38^a	1.19±20.54^a	1.23±17.30^a	1.19±16.39a	1.22±18.62^a	.423	.863
Protein (mg/dL)	2.90±0.81^a	3.06±0.68^a	2.82±0.88^a	3.15±0.87^a	3.08±0.56^a	3.22±1.07^a	3.12±0.75^a	.870	.518
Cholesterol (mg/dL)	1.39±14.43^a	1.50±64.48^a	1.39±14.58^a	1.56±54.43^a	1.38±15.28^a	1.41±19.82^a	1.42±16.66^a	1.180	.318
Urea (mg/dL)	22.53±2.71^a	22.36±2.67^a	22.43±2.78^a	22.63±2.79^a	23.13±3.21^a	23.10±3.20^a	22.96±2.25^a	.391	.884
Triglyceride (mg/dL)	88.80±11.72^a	90.20±15.49^a	87.80±12.43^a	94.00±20.77^a	84.90±11.67^a	89.03±11.88^a	87.13±12.97^a	1.192	.312
Uric acid (mg/dL)	1.83±0.38^a	1.86±0.44^a	1.95±0.46^a	2.01±0.59^a	2.00±0.66^a	2.01±0.76^a	1.94±0.54^a	.469	.831

RBC, red blood cells; PDW, platelet distribution width; MCV, mean cell volume; HB, haemoglobin; PLT, platelet; MPV, mean platelet volume; TLC, total leukocyte count; LYM, lymphocytes; NEU, neutrophils; EOS, eosinophils; MO, monocytes; BAS, basophils; PCV, packed cell volume.

DISCUSSION

It has been considered that clinical chemistry is in the reptiles and other lower vertebrate species is representative of mammals. Our findings have been approved the impacts of physiological and environmental factors on the freshwater turtle species based on hematological and blood biochemistry parameters.

Our analysed parameters appeared to be important health tool to predict the capability of general condition of turtle species in assessing the hematological and blood biochemistry parameters (Whiting *et al*., 2007; Oliveria-Junior *et al*., 2009). Glucose level in the blood (Lutcavage *et al*., 1995), and the total protein levels (Whiting *et al*., 2007) have also been associated with the nutrients and nutritional status of turtle species. Generally, the hypoglycemia is associated with the malnutrition, starvation, acute liver disease and more commonly to the septicaemia (Campbell, 1996). The hypoproteinaemia is caused due to intensification in the protein loss or happen when the animal body is incapable to produce enough protein (Swimmer, 2000). It may also be due to intake of lower level of crude protein (Lutacavage *et al*., 1995). In the *C. mydas*, the starvation caused shortened levels of plasma glucose (Bonnet, 1979). It has been argued that the total protein concentration is a best sign for the nutritional status because when a decrease in the level of crude protein intake is identified (Whiting *et al*., 2007). It has been observed that the concentration of plasma urea is normally higher than protein level or increased protein intake which may be either through the nutritional protein or increased due to a tissue breakdown (Whiting *et al*., 2007). Moreover, it is recognized that the chelonians are extremely stronger animals and could be generally active even when they suffered acute anaemia and hypoproteinaemia (Norton, 2000). However, the lipid metabolism disturbance can be assessed through the cholesterol level changes, which is secreted from the liver in the form of bile acids (Swimmer, 2000). It has been biochemically characterized that lipids have effective mechanism for energy storing to be used for the conservational purposes (Christopher, 1999; Derickson, 1976). The availability of food is considered that the deposition of lipids quantity when the lipids are stored, and these lipids are stored for the variety of purposes

(Derickson, 1976). The level of metabolic depression is an important strategy for the survival in the numerous animal species under different environments for the deprivation of prolonged nutrition such as starvations and hibernations (Storey and Storey, 2004; Makareiva *et al*., 2006).

In the group of chelonians, the red blood cell range can be counted as a comparatively index for the occurrence, nourishment or generally animal health (Campbell, 1996; Petersen, 2002; Whiting *et al*., 2007; Oliveira-Junio *et al*., 2009), because it is major cause of anaemia which is directly affected by the chronic poor nutrition or related with the consumption of protein (Christopher, 1999; Peterson, 2002). In the reptiles, the range of haemocratic less than 20.0% is indicator an anemia (Christopher, 1999).

For calculating physiological disruptions in chelonians, blood parameters are useful. They can thus provide important information for disease diagnosis and prognosis (Oleivera-Junior *et al*., 2009). Hematological analyses are useful for animal health and disease control and for clinic and conservationist differentiation of the physiological cycles. These are also valuable to create hematological reference values for turtles and to generate a reliable baseline of clinical laboratory data for turtles in relation to sex and season. We have analysed the data using repeated variance analysis (P<0.05) (Chung *et al*., 2009). Hematology is a detailed investigation of the numbers and morphology (shape and size) of blood corpuscles including erythrocytes, leukocytes and thrombocytes and the interpretation of these results in the diagnosis and monitoring of nutritional deficiencies and certain pathological conditions. Hematological tests provide useful reptile health information including freshwater turtles (Chung *et al*., 2009). Safety and disease detection in turtles is problematic in contrast to other animal species. Biochemical and hematological plasma variables are critical for managing endangered freshwater turtles (Casal *et al*., 2009).

In the present study, concentrations of RBC, PLT, and uric acid were observed for *Pangshura tecta,* and PDW, MPV, TLC, and PCV, protein, cholesterol, and triglycerides were observed higher in *Nilssonia hurum*. The concentration of MCV was observed higher in *Chitra indica*, while the concentration of HB and LYM was observed higher *Chitra indica*. The concentration of NEU was observed higher in *Kachuga smithii* and the concentration of EOS was observed higher in *Hardella thurjii*. The concentration of BAS was observed zero in all studied population of freshwater turtles. The concentration of MO was observed higher in *Hardella thurjii* and the serum concentration of glucose was observed higher in *Kachuga smithii*. The concentration of urea was observed higher in *A. gangeticus* species of turtle.

The eosinophil volume was assessed different based on Tukey-HSD test. *Lissemys punctata* as differ from *K. smithii*, *A. hurum* and *H. thurjii* while others were similar and there was no significant difference (P< 0.05) among them. The *A. ganeticus* as different from *K. smithii*, *A. hurum* and *H. thurjii* while others were similar and there was no significance difference (P< 0.05) amongt them.

The concentration of monocytes was different based on Tukey- HSD Test. The *Lissemys punctata* was differt from *A. gengeticus*, *Chitra indica*, and *K. tecta* while others were similar and there was no significant difference among them. The *A. gangeticus* as different from *Lissemys punctata* and *H. thurjii* while others were similar and there is no significance difference (P< 0.05) between them.

It has been shown that higher variability in the size and range is plausible when the blood profile was compared among freshwater turtles in Pakistan. Generally, the species of *E. orbicularis* show a mean values of L/W ranging from 1.6 to 1.8 μm^2, with the average cell ranging from 11.9 to 225.1 μm^2. In the species of *E. trinacris*, the range of red blood cells were found larger when compared with the erythrocyte cells in freshwater turtle species from the studied area of Indus River, Guddu, Sindh, Pakistan. The range of nuclei of the red blood cells were smaller in size in the studied turtle species. The morphology of leukocyte cells showed they have variance in their shape, size and range when compared with other habitat of turtle species. Most of herpetologists agree on this point that the reptilian species don't have present neutrophils as well as heterophiles and eosinophils, both of them showed acidophiles granules (Canflied, 1998). Few studies on the acidophils classification (i.e., therehils and eosinophils) are considered as single type of cell during the different maturation phases (Azevedo and Lunardi, 2003) while the neutrophils cells have been reported only in few studies (Wood and Ebanks, 1984; Pitol *et al*., 2007). Various studies have shed the light on the analysis of leukocyte cells under the light microscope in the species of *Podocnemis expansa* (Schwaeiger, 1812). A study has reported basophils, eosinophil, lymphocytes, monocytes and heterotrophilis (Metin *et al*., 2006) in *Emys orbicularis*.

We believe that in turtles there are two types of eosinophils different from each other on the base of cytoplasmic granules shape. According to Azevedo and Lunardi (2003), the blood of *Chrysemys dorbigi* there are two different types of granulocytes and eosinophilia with different type of elongated cytoplasmic granules (Dumberil and Bibron, 1835). It is believed that the eosinophils have different maturation phases, but they have different cell types; i.e., eosinophils and heterophils (Azevedo and Lunardi (2003)). The most of leukocytes in *E. trinacris* were identified as heterophils, basophils and eosinophils

and same findings were also reported in *Podocnemis expansa* (Oliveira-Junior *et al*., 2009). However, these studies have reported these differences in other turtle species. Moreover, only captive female individual of *Clemmys muhlenbergii* carry higher eosinophils count and higher eosinophils percentage related with the captive males despite the wild females were not differt from the wild males (Brenner *et al*., 2002).

The basophils were reported plentiful leukocyte type in *Graptemys gibbonsi* where the total leukocytes was found to be 40% (Perpinan *et al*., 2008). In the group of *Chelonia*, the higher percentages of basophils (50-63%) were reported in *Chelydra srepentina* (Linnaeus, 1758). In contrast, the higher percentage of basophils were reported in different species such as 5.7% in *Gopherus polyphemus* (Daudin, 1802; Taylor and Jacobson, 1982) and 80% in *Geochelone radiate* (Shaw, 1802; Marks and Citino, 1990). In the species of *Clemmys muhlenbergii,* the number of basophils were missing (˜0.8 for both sexes) as described earlier (Brenner *et al*., 2002). In the current study, the numbers of basophils and leukocyte types in all turtle species from the Indus River, Guddu were missing. Generally, the monocyte cells were not recognized in *Chelonia mydas* (Linnaeus, 1758) as has been argued earlier (Wood and Ebanks, 1984; Aguirre *et al*., 1995). Moreover, the monocytes are not detectable if the blood smears are found with blood which was existed eight or more hours earlier (Work *et al*., 1998). The shape of monocytes in *E. trinacris* were similar with the *E. orbicularis* (Metin *et al*., 2006) or *Ocadia sinensis* (Gray, 1870, Chung *et al*., 2009). The shapes of monocytes were round in *E. trinaccris* and had a similar size in both males and females (11.3 and 11.7 µm). Their presence was same for both sexes (~ 4.2%). In our findings, it was confirmed that the concentration of leukocyte was presents in all freshwater turtle species.

CONCLUSION

The present study provides baseline values for the hematology and serum biochemistry of freshwater turtles of the river Indus, Guddu, Sindh, Pakistan, and can be used as reference values for the assessment of diseased health conditions in the same species. The results of this study provide quantitative evidence on the biochemical hematology and serum parameters of freshwater turtles (Order testudines) as a model for comparative physiology of freshwater turtles. In conclusion, our findings contribute to the growing information on the hematological and biochemical profile of aquatic species in the territory of Indus River, Guddu Barrage. These finding can be applied to classify the blood profile counted for the lower nutrition in the natural environments. However, it is concluded that the examinations of hematological and biochemical parameters are best indicators of physiological status, except the number of thrombocytes. Thus, these parameters of blood profile may be successfully applied for examining the nutritional and health status of wild animals of freshwater turtles. Furthermore, these finding must be consummate as a major tool for health status of the wild population as a major part for the necessary veterinary inspection on the framing of turtles in Pakistan. These outcomes could also be generalized for the monitoring of projects for these species in the wild ranging, which are more vulnerable due to high demand of meat consumption as well as production of eggs. If this objective is given high priority by the governmental organizations, a species conservation plan may succeed in the natural environment of Pakistan.

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IRB approval

This research study was approved by Institutional Review Board (IRB) of University of Sindh Jamshoro. There is not any potential conflict of interest observed by the authors.

Ethical statement

This research study was approved by the Animal Ethical Committee of University of Sindh Jamshoro. However, all these turtle species were captured in the wild and were kept under the normal condition, which were provided with a suitable environment to minimize the animal stress. There is not any potential conflict of interest observed by the authors.

Statement of conflict of interest

The authors have declared no conflict of interest.

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