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Tigers Blood: Haematological and Biochemical Studies

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1. Introduction

Tiger (*Panthera tigris tigris*) population in their historic ranges is critically endangered owing to habitat destructions, ruthless poaching and retaliatory killing. The tiger population now remains in few thousands located in about 150 fragments in 13 countries (Karanth and Gopal, 2005). However, declination is also associated with health related problems such as nutritional deficiencies and infectious diseases (Prater, 2005). Therefore, health monitoring and scientific health management, disease diagnosis and treatment should be made mandatory for conservation of wildlife as the tiger is a key stone species and important member of forest ecology (Shrivastav, 2001).

Haematological and biochemical studies are important tool for health evaluation and their interpretations to know the status of physiological functions of various organs. The concentration of biochemical constituents in tissues as well as in body fluid is fixed and during adverse conditions, it may be elevated or decreased (Douglas and Nelson, 1991). However, qualitative and quantitative analysis of corpuscles and chemical constituents of plasma or serum are closely linked with functional unit of the cell and their assessments may reflect the physiological disorders (Harvey, 1997).

Nevertheless, several factors involved to transmit infectious diseases either mechanically or biologically through contaminated water, food or vectors (Lice, Flea, Ticks and Mites) and the pathogens may alter the normal physiology (Shah, 1983). Viral, bacterial and parasitic diseases are very common in tigers which can affect the haematological and biochemical normal values (Rao and Acharyjo, 2002). Types of anaemia and significant blood loss may be estimated through complete blood count (CBC) and physiological function of different organs by biochemical parameters (Jain, 1986). Qualitative and quantitative reduction in the blood commonly observed in captive felid particularly in cubs those maintaining on milk alone. The values of liver function test, elevated on repeated immobilization by sedative

drugs. It has been experienced that the values of serum enzymes increased after 72 hrs interval of 2nd immobilization by Ketamine and Xylazine mixture (personal communication, Shrivastav 2012). Sign of anemia such as pale mucous membranes weakness, fatigue and tachycardia may be observed depending on the severity of anemia. A variety of abnormalities may be noticed by analysis of blood, bone marrow cytology, serum chemistry and urine analysis.

Wild felids are commonly injured in territorial fight or sometimes serious injuries and internal hemorrhages occur during hunting. If blood loss is above the 50% of total volume in short period may be fatal and tiger may die due to hypovolemic shock. Information on haematology and blood biochemistry is meagre in wild animals. However, several studies on selected haematological parameters of exotic species of captive Felids have been reported. Currier and Russell (1982) studied the higher pack cell volume in wild and captive mountain lions (*Felis concolor*) and Fowler (1986) has reviewed the haematological and biochemical profile of Felids including captive tigers whereas Jain (1986) reviewed the information of the genera *Panthera*, *Felis*, *Uncia* and *Acinonyx* concluded that blood parameters were almost similar to that of domestic cat with exception of higher concentration of plasma protein and Pack cell volume (PCV). Seal et al. (1987) have studied the haematological and biochemical profile of captive Bengal tigers with emphasis of anaesthetic effect on blood parameters. Chandranaik et.al. (2006) also studied the haematology of physically restrained tigers that were kept in squeeze cages without using anaesthetics. However, the haematological and biochemical studies were made in twelve apparently healthy tigers in free ranges of Central India (Shrivastav et.al. 2011).

Health monitoring, assessment of health during treatment and disease diagnosis in free range tigers needs baseline data on haemato-biochemical parameters. This baseline data is important especially for comparative health assessment of felids during outbreaks of diseases between sylvatic and domestic cycle vice versa. It is also required, as the tiger is on top of the sylvatic food chain and to be protected for maintaining balances in ecosystem (Gopal, 1993).

2. Blood collection and investigation

The collection of blood for laboratory investigations is comparatively difficult in both free range and captive tigers and only possible when animal is sedated or restrained properly in squeeze cage.

Withstanding facts, chemical capture is comparatively safe, if accomplished by trained and experienced wildlife veterinarians. There are several drugs available for sedation. Each drug works in a different manner and is more suited to some species only. The time required for a drug to have an effect depends upon the factors such as route of administration, absorption rate, concentration and physiological status of the animal while it is difficult to generalize the choice of drug and doses (WIL, 1985). It depends upon circumstances like species of the animal, age, sex, weight, location, temperature regimes in season, time of the day and

emotional state. Shrivastav et.al.(2011) have used Xylazine hydrochloride + Ketamine hydrochloride as sedative drugs with the help of Tel-inject projectile syringe to immobilize the tigers of free range while Yohimbine hydrochloride was used as reversal drug.

Prior to collection of blood from immobilized animal, it is an essential protocol to obtain normal values only through free flow of the collected blood drawn from the animal either at rest or under conditions of least excitement to minimize the physiological variations in cell count (Jain, 1986). Normally the cephalic saphenous, femoral and jugular veins are used for collection of blood from dog, cat and non human primates while in tigers these sites are not convenient because the blood collector remained in front of face of the tiger. The caudal vein is convenient and safer site for blood collection (Shrivastav et.al.2011).

From sedated free range tigers, 2-5 ml of blood is drawn by venipuncture of the caudal vein through 18 no gauge disposal syringe in a tube containing Ethylenediaminetetraacetate (EDTA at 2 mg/ml of blood) as the anticoagulant (Shrivastav et.al. 2011). The blood samples should be processed as soon as possible after collection. If a delay is anticipated, it should be refrigerated at 4°C (Jain, 1986).The blood sample should be mixed several times before a portion is removed for test procedure (Shrivastav and Sharma, 2000). Automatic devices providing a continuous rocking or circular motion have been found satisfactory, but prolonged mixing should be avoided, particularly on a device with circular motion, to prevent a mechanical trauma to various blood cells, especially erythrocyte. In any event, blood smear must be made immediately after blood collection, either directly from fresh blood or after anticoagulation. Blood films should be dried quickly and protected from dust and flies till stained (Shrivastav and Sharma, 2005). Blood films can be made on glass slides and on cover slips.The haematological analysis needs precautionary measures and blood smear is stained with Romanowsky stains and at least 200 white cells should be examined for the differential leukocytes count. Simultaneously, the blood smears must be screened for parasitic blood protozoa, flagellates and rickettsial infections.

3. Haematology of Tiger

3.1. Erythrocytes

The morphology of erythrocytes varies with 2 to 7.6, $7.3 \pm 0.45 \mu\text{m}$ in size; appears circular, discoid, central pallor with slight anisocytosis whereas the rouleaux formation(Plate- 2) is common in tiger's blood. Chandranaik, et.al. (2006) also reported the mild anisocytosis in physically restrained tigers. However, the range and mean (with one standard deviation) of total erythrocyte count (TEC) was 4.66 to 9.15, 7.9 ± 1.42 million / μl . Likewise haemoglobin concentration (Hb) was obtained 9.8 to13.5, 12.8 ± 1.65 mg/dl in male and 7.8 to11.5, 10.8 ± 1.05 mg/dl in female tigers (Shrivastav et.al. 2011).

Jain (1986) defined that the rouleaux formation is associated with erythrocyte sedimentation rate (ESR) and useful for evaluation of the disease status. Shrivastav et. al. (2011) encountered ESR (14 to 26, 21 ± 4.21 /hour) and PCV (36 to 45, 38 ± 4.45 %) in free range tigers (Table 1). The consequences of ESR and PCV up and downs mostly confined to

erythrocyte osmotic fragility that increased in case of immune-mediated hemolytic anemia. Taketa et. al. (1967) have assessed the oxygen affinity of the haemoglobin is much lower in felines than that of other mammals including humans.

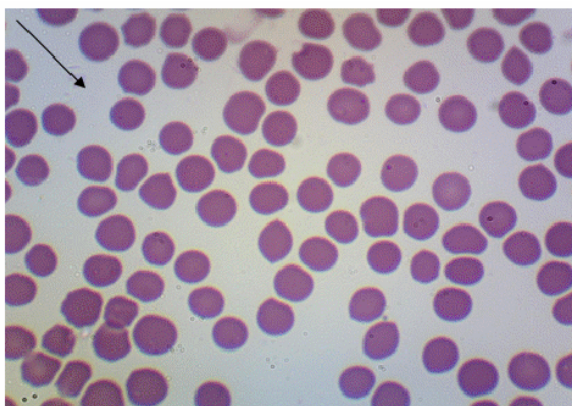
	Haematology	Unit	Range	Mean	SE (±)
1	Red blood corpuscles	(TEC) ×106/μl	4.66–9.15	7.90	1.42
2	Total Leukocytes Count	(TLC) ×103/μl	6.2–11.05	8.50	1.42
3	Haemoglobin	(Hb) g/dl	7.8–13.8	12.8	1.65
4	Haematocrit	(PCV) Ratio	36–45	38	2.54
5	Erythrocyte sedimentation rate	(ESR) Hours	14–26	21	4.21
6	Icterus index	(II) u/l	2– 5	2	1.51
7	Differential leukocyte count	%			
i	Neutrophils –		57–75	60	5.08
ii	Lymphocytes –		18–35	30	4.56
iii	Monocytes –		2– 6	0.5	1.21
iv	Eosinophils –		2–6	0.4	1.30
v	Basophils –		0–4	0.1	1.21
	Blood plasma biochemistry				
1	Albumin (ALB)	g/dl	2.1–4.6	3.50	0.99
2	Total protein (TPROT)	g/dl	3.7–8.7	6.40	1.88
3	Total bilirubin TBIL)	mg/dl	0.4–3.2	1.90	1.21
4	Creatinine (CRE)	mg/dl	1.6–4.6	2.90	1.03
5	Blood urea nitrogen (BUN)	mg/dl	6.5–48.2	27.90	13.77
6	Alanine Aminotransferase (ALT)	IU/L	21.2–109.0	67.88	27.84
7	Aspartate Aminotransferase (AST)	IU/L	14.4–84.0	57.96	17.27

Table 1. Haematological and Biochemical Values of Bengal tigers (*Panthera tigris tigris*)

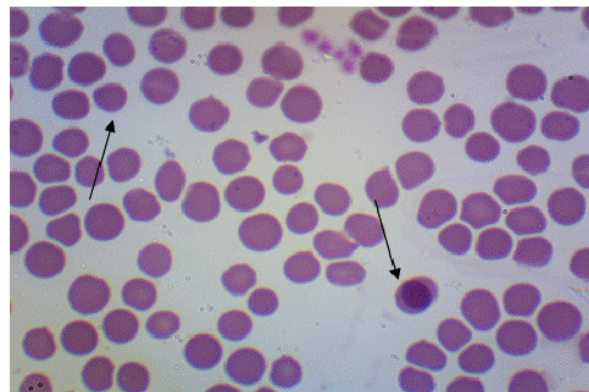
Jain (1986) reviewed the haematological parameters of big cats including *Panthera*, *Felis*, *Uncia* and *Acinonyx* and found that the blood composition were almost similar. Among all cats few erythrocytes had single refractile structure (Heinz body) when stained with new methylene blue stain. The Heinz body appearance in erythrocytes is the unique feature of the family Felidae (Plate -1) while they are not visible usually in blood films with Romanowsky stain (Jain,1986). The reduction in erythrocyte count (TEC) and haemoglobin concentration (Hb) are generally associated with anaemia and classified on the basis of erythrocyte morphology,

pathogenetic mechanism and bone marrow erythroid response (Jain, 1986). In wild animals the clinical signs and their magnitude depends on habitat and availability of nutritive materials. Prolonged nutritional deficiencies of protein vitamins and minerals essential for erythrocytes production lead to anaemia. The type of anaemia varies with the nutritional deficiency, blood loss and the animal species involved. Despite the nutritional consequences the blood loss may be encountered through traumatic injuries, complication in blood vascular system, thrombocytopenia, and coagulation disorders. A normocytic – nonchromatic, non responsive anaemia is commonly found in association with chronic infections, chronic infectious inflammatory conditions and some type of malignancies though microcytic-hypochromic is the sign of iron deficiencies (Jain and Kono 1975).

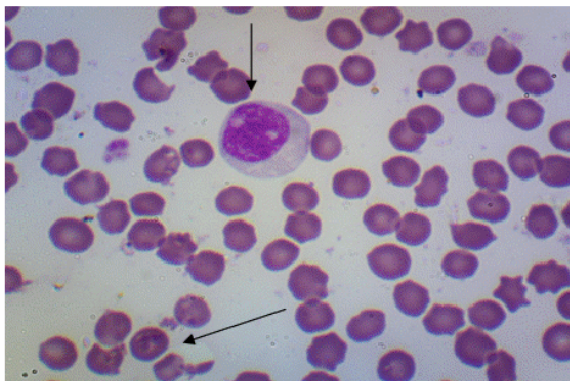
Several blood sucking parasites produce blood loss anaemia in tigers like Ancylostomes, Toxoscaris that may cause haemolytic anaemia while Trypanosomes, Babesia and Haemobartonella (*Mycoplasma haemofelis*) may alter the total blood as well as plasma volumes with acute blood loss. Chronic blood loss may lead to gastrointestinal lesions, ulcers, heavy parasitism like coccidiosis, neoplasm with bleeding into body cavity, deficiency of Vitamin K and prothremin etc.



A: Erythrocytes



B: i. Anisocytosis ii. metarubricyte (nucleated RBC)



C: i. Heinz Bodies ii. Lymphocyte ↓



D: ↑ i. Poikilocytosis ii. Lymphocyte ↓

Plate 1. Tiger Blood smear stained with Modified Wright Stain x1000.

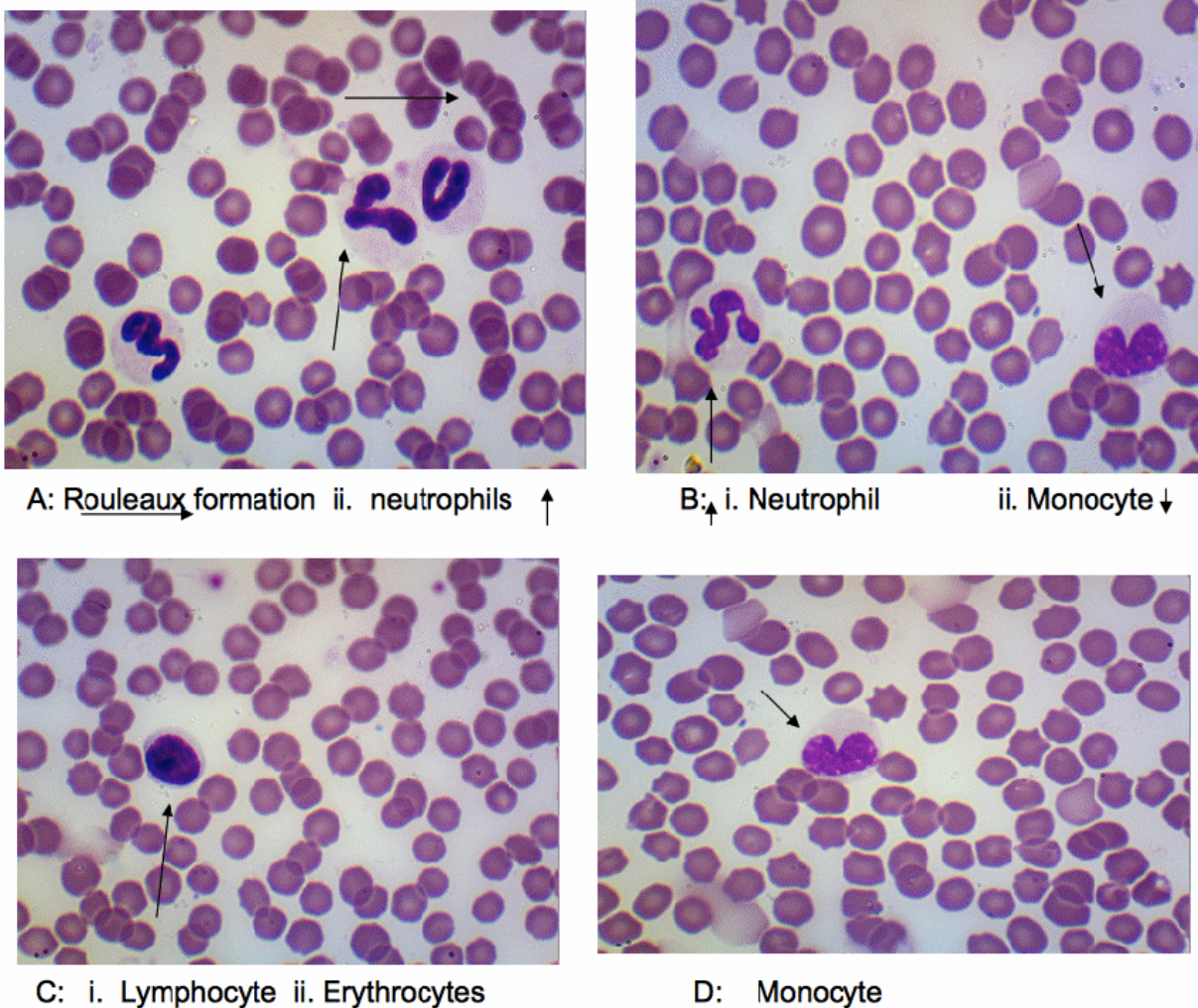


Plate 2. Tiger Blood smear stained with Modified Wright Stain x1000.

4. Leukocytes

The total leukocytes count (TLC) and differential leukocytes count are important parameters to judge the body response against diseases. The TLC was 6.2 to 11.05, 8.5 ± 1.42 thousand/ μl in free range tigers while differential leukocyte counts (DLC) reflect the information of infectious manifestations. A leukocytosis may be physiologic mediated by endogenous release of epinephrine or corticosteroids or it may be pathologic response to a diseases process (reactive leukocytosis) or a result of a neoplastic change in the haematopoiesis (proliferative leukocytosis) while leucopenia is always pathologic event. Quantitative and qualitative changes in a particular type of leukocyte indirectly reflect the nature of disease process and the body response to it.

Jain (1986) reported physiologic factors such as fright and “emotional” disturbances as an immediate effect on TLC and DLC and may confined to interpretation of conditions. The normal response to the stress is decrease in lymphocytes and eosinophil numbers. In “emotional” leukocytosis, lymphocyte numbers are increased and equal or exceed

neutrophil numbers while eosinophil commonly not affected. Meyers-Wallen et. al. (1984) observed the young cats normally have high lymphocyte counts and hence a greater tendency to develop lymphocytosis than the adults. This observation may also be attributed in the case of tigers as they belong to the member of same family with wild habitat as an escape behavior. Increases in neutrophil numbers due to physiologic influences are more pronounced in felines than in canines because of the difference in the intravascular distribution of neutrophils. Prasse et. al.(1973) have observed 3 times mean marginal pool of neutrophils of clinically healthy cats than the circulating pool whereas in dog it was about equal or slightly greater.

4.1. The neutrophils

Neutrophils considered as first line of defense against microbial infections and are important participants in inflammatory reactions. Shrivastav, et.al. (2011) encountered 57 to 75, 60 ± 5.08 % with multi-lobed nuclei of 3-5 lobes while sometimes mono-lobed nuclei with pale to slightly pink granules in the cytoplasm in free range tigers(Plate3). Chandranaik, et.al. (2006) has also reported the segmented or multi lobed nuclei while Jain (1986) studied the sex chromatin in few neutrophils as the drumstick lobe in the female cats.

The changes in blood neutrophil differential count (Haden, 1935) is associated with many consequences related to infectious diseases. Several functions have been suggested for the contents of granules, as neutrophils are phagocytic cells and regulating adhesiveness and aggression hydroxyl radical formation and generation of compliment derived chemotactic factors while azurophilic granules are involved in modulation of inflammatory process (Gallin, et. al.1982). Condensation of nuclear chromatin leads to formation of darker-staining plaques separated by delicate, light-staining areas with slight brown colour cytoplasm.

4.2. The eosinophils

Shrivastav et.al. (2011) observed eosinophils contained small, uniformly round bright eosinophilic granules almost occupying the entire and clear cytoplasm (Plate 3). These cells were encountered 2 to 6, 4 ± 1.21 % in free range tigers (Table 1). The nuclei of the cells were generally less lobulated than those of the neutrophils. The eosinophils are slightly larger than neutrophils. Chandranaik, et.al. (2006) also observed the larger eosinophils larger than neutrophils and lobulated nuclei with orange cytoplasm in tigers. Jain (1986) reported the granules of the eosinophil are rod-like in domestic cats and Cheetah (*Acinonyx jubatus*) while round granules in the eosinophils of Lion and Leopard. The eosinophils are commonly seen in prolonged parasitic infections or allergic disorders.

4.3. The basophiles

The basophile is a numerically insignificant but functionally important leukocyte that resemble with mast cells and it believed to share similar function as it is associated with allergic reaction, inflammatory process and immunocompantancy to the body fluids. Galli et.

al. (1982) reported basophiles of cats have a limited capacity to phagocytised. Chadranaik et.al. (2006) have reported smaller basophiles than eosinophils with pale lavender pink stained cytoplasmic granules in physically restrained tigers. Jain (1986) observed the mature basophiles contains numerous small, round, lightly stained (pinkish or orangish) granules in light gray cytoplasm in experimental cats. The basophiles were rarely observed up to the size with 0 to 0.4 0.1 ± 1.21 5% in free range tigers.

4.4. The lymphocytes

The lymphocytes are comparatively smaller than eosinophils with round to oval nucleus occupying most space with spherical nucleus (Plate 3). Small and large lymphocytes were also seen in the blood smear. Some lymphocytes contained a few azurophilic granules in their cytoplasm. Jain (1986) reported small lymphocytes is common in cats with patchy nucleus and dense clumps of heterochromatin. In tigers, Shrivastav et.al.(2011) have report lymphocytes from 18 to 35 , $30 \pm 4.56 \%$ (Table 1& Plates 2).

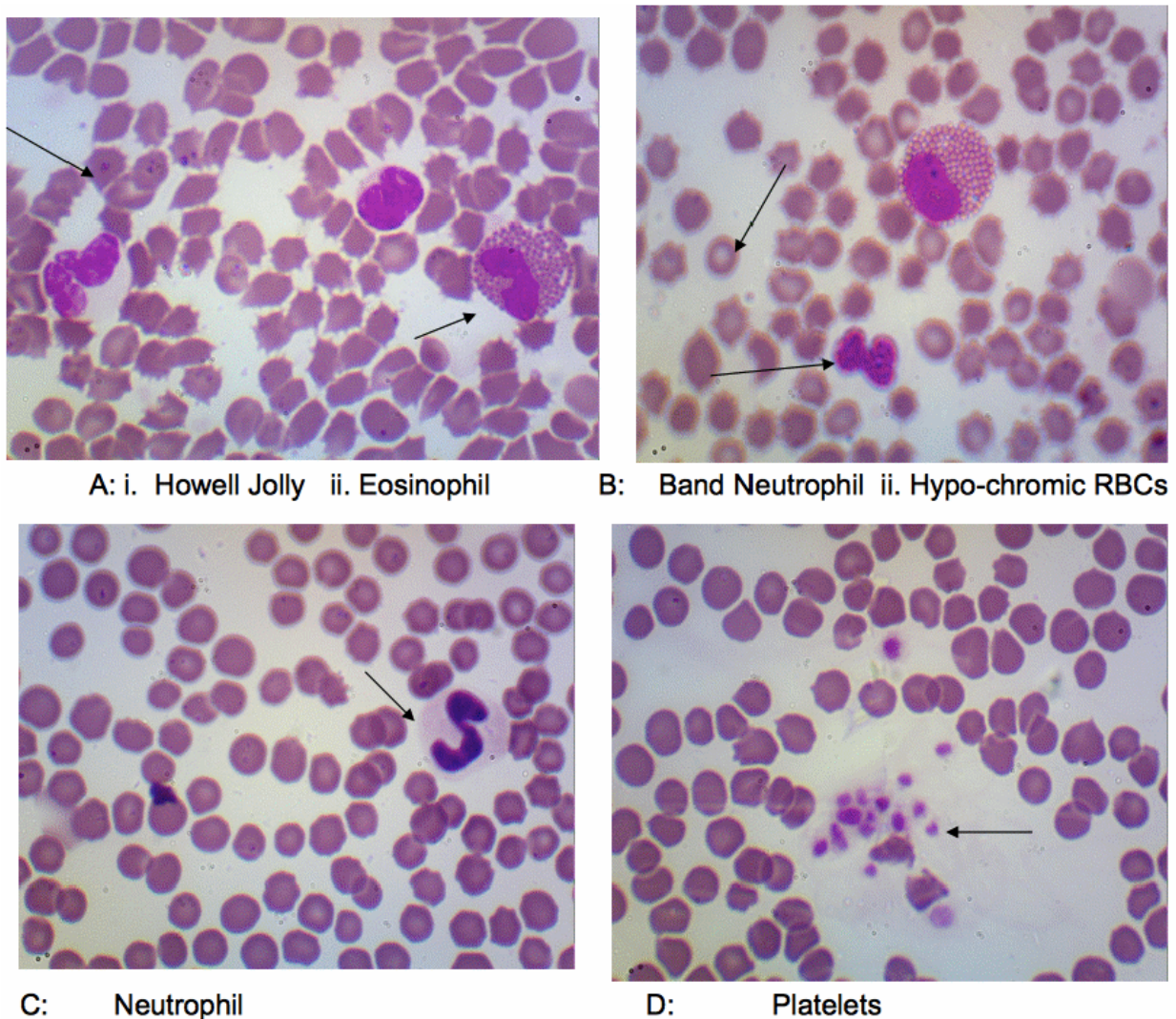


Plate 3. Tiger Blood smear stained with Modified Wright Stain x1000.

4.5. The monocytes

The monocytes are usually larger than lymphocytes. Shrivastav et.al. (2011) encountered 2 to 6, $5 \pm 1.21\%$ monocytes in free range tigers with distinguishing feature of the reddish grey nucleus and well defined vacuoles, the nucleus of the monocytes reported amoeboid and some time noticed horseshoe shaped nucleus while cytoplasm stained slightly blue and appeared foamy – vacuolated. (Plate 3). Jain (1986) has also observed similar monocytes in experimental domestic cats.

The monocytes are associated with phagocytosis principally against intracellular bacteria, viruses, fungi and protozoa. The cells perform regulation of the immune response, phagocytic removal of tissue debris (affected cells, antibody coated cells and other foreign materials) as scavenger (Jain, 1986).

Rao and Acharjyo, (2002) have emphasized that macrophages, B-lymphocytes and bone marrow precursor cells are targeted cells for viral replication and commonly observed in Feline Pan-leucopenia (FPL), Feline Viral Rhinotracheitis (FVR), Immunodeficiency Syndrome (FIDS), Canine Distemper (CD) and Inclusion Body Hepatitis (IBH) etc. The body immune system is badly affected and gradually reduced.

4.6. The platelets

Platelets are abundant in blood smear and usually distributed in small to large clumps. Shrivastav et.al.(2011) reported that individual platelets are pleomorphic with rounded to elongated shapes with a central cluster of azurophilic granules (Plate 3). Jain (1986) has observed the clumping platelets in cat blood and emphasized that the platelets of the cats clump readily during excitement of 3 minutes caused a sudden increase in platelet counts. A slight decrease occurred in sympathectomized cats and a somewhat greater decrease reported in splenectomized cats.

4.7. Blood biochemistry

The concentration of biochemical compounds in tissues and body fluid can be measured in a colorimetry, as it is capable of absorbing light of a particular wave length (Singh, 2004). Thus the health status of animal can be assessed by evaluation of Blood gases, acid base balances, electrolytes, metabolic intermediates, inorganic ions, enzymes and hormones.

Shrivastav, et.al. (2011) have conducted blood biochemical analysis of free range tigers for Albumin, Total protein, Total bilirubin, glucose, creatinine, Blood urea nitrogen (BUN), Glutamic oxalo-transaminase (GOT/AST), Glutamic pyruvic transaminase (GPT/ALT) by using an ERBA Chem-5 plus auto-analyzer (Transasia Bo-medicals Ltd.) with standard ERBA reagent kits for respective plasma constituents. The statistical analysis of obtained data is expressed in range, mean and standard deviation.

4.8. Icterus index

Jain (1986) reported an increase in the values of Icterus index in plasma is an indicative of an absolute increase in bilirubin concentration due to removal of aged erythrocytes from the circulation by the reticuloendothelial and liver. Shrivastav, et.al. (2011) reported 2 to 5, \pm 2.1.5 units. in apparently healthy tigers of free range.

4.9. Total plasma protein

Protein in plasma can provide information reflecting functional status of various organ and systems as blood is composed of approx 20 % of protein excluding haemoglobin. However, the total protein values gives the information on nutritional consequences or severe organ diseases as they transported the carrier of most of the constituents of the plasma, maintains the colloid osmotic pressure, act as catalysts in biochemical reaction and play important role in formation of fibrin polymers during clot formation (Richard, 1991). The total plasma protein in tigers was estimated 3.7-8.7 to 6.4, \pm 1.88g /dl. The values are commonly increases in haemoconcentration and reduced in malnutrition, hepatopathy, less intake of protein and in neoplastic condition etc.

4.10. Plasma albumin

The liver produces all the albumin and globulins while a small amount of globulins is produced by reticuloendothelial tissue (Benjamin, 1979). Liver synthetic capacity or protein-losing nephropathy can be measured by albumin estimation in the blood plasma or serum. It also can interpret high or low calcium and magnesium level since albumin binds about one half of each of the ions (Richard, 1991). However, it appears to be a direct correlation between albumin turnover and body size because it is clinically significant. It is usually constituted with two third of total plasma protein and also serve as mobile amino acids for the liver (Mc Pherson, 1991). Generally hypoalbuminism is observed in malnutrition, increased protein catabolism, nephropathy and chronic enteropathy. Shrivastav et. al. (2011) reported plasma albumin level 2.1 to 4.6, \pm 3.5 g /dl, in free range tigers. Reduction in total albumin values is observed in malnutrition, liver diseases, stress, kidney dysfunction etc.

4.11. Total bilirubin

Bilirubin is a breakdown product of heme about 70 percent of which is derived from senescent red cells (Crawford et. al., 1988) however, 15 percent comes from hepatic cytoplasm and mitochondrial cytochromes and some from renal and other cytochromes, and some from defective red blood cell broken down in the bone marrow before release. Shrivastav et. al. (2011) reported 0.4 to 3.2, 1.90, \pm 1.21mg /dl, total bilirubin in free range tigers. The yellow color of serum or plasma is due chiefly to the pressure of bilirubin. Increased concentration of bilirubin is commonly seen in haemolysis hepatocellular damage, biliary obstruction prolonged fasting reduced intake fluids etc.

4.12. Creatinine

Creatinine is important in muscles metabolism in that it provides storage of high energy phosphates through synthesis of phosphocreatine (Benjamin, 1979). It was estimated in tigers as 1.6 to 4.6, 2.9, ± 1.03 mg /dl. Serum or plasma creatinine concentration and urinary creatine secretion are increased significantly by skeletal muscles necrosis or atrophy and defect in renal functions (Pennington, 1971).

4.13. Blood urea nitrogen

Urea is the end product of protein and amino acids and is generated in the liver through urea cycle (Woo and Cannon, 1991). Blood Urea Nitrogen is one of the important tools to know the renal function status. The values of BUN (6.5 to 48.2, 27.9, ± 13.7 mg /dl) was observed in free range tigers is commonly seen in malnutrition and hepatic insufficiencies, however, increased BUN is generally associated with renal disease congestive heart failure, shock, hypertension etc. Shrivastav et. al. (2011) observed the high rise might be also due to adlib intake of meat as the Royal Bengal Tiger can consume 35-40 kg meat of pray animal at a time (Prater, 2005).

4.14. Hepatic enzymes

The serum enzymes used routinely in clinical diagnosis are synthesized in liver (Schaffner, and Schaffner, 1991). In hepatocellular or in cholestatic forms of liver injury these hepatic enzymes are released in to the serum. The serum enzyme activities that are elevated in hepato cellulardamage are Alanine Aminotransferase (ALT) Aspartate Aminotransferase (AST) Ornithine Carbamoyltransferase (OCT), Glutamic Dehydrogenase (GD) Sorbitol Dehydrogenase (SDH) and arginase. The elevated serum activities that suggest cholestasis (intra hepatic or extrahepatic) are Alkaline phosphatase (AP), Gamma glutamyl transpeptidase (GGT) and 5' nucleotidase (5'ND). The pathogenesis of the hepatic disease in carnivores especially in Felids are associated with viral hepatitis, parasitic infections or mechanical injuries (Rao and Acharjyo, 2002). The liver has great functional reserves and signs of hepatic failure often do not develop until 70% or more of the functional capacity of the liver is lost (Tennant, 1997).

4.15. Alanine aminotransferase (ALT)

Alanine Aminotransferase (ALT) was also termed as SGPT and used by many estimations and large number are found in Hepatocytes in cats, dogs and primates (Benjamin, 1979). The ALT was estimated 21.2 to 109.0, 67.9, 27.84 \pm IU /L in free range healthy tigers (Shrivastav et. al, 2011).

4.16. Aspartate aminotransferase (AST)

Apart from liver, AST (Aspartate Aminotransferase) is also present in muscles and cardiac muscles. The higher value of AST though is not an organ specific but used as an indicator of

liver dysfunctions. Shrivastav, et. al. (2011) reported 14.4 to 84.0, 57.9 $17.27 \pm$ IU /L in the free range tigers.

The haemato-biochemical profile of the Bengal tigers reported by Shrivastav et. al. (2011) was compared with the values of captive Bengal tigers (Seal et al. 1987), and no major differences were noticed except in ALT, AST and BUN. The mean values (BUN (27.90 ± 13.77 mg/dl), ALT (67.80 ± 27.84 IU/L) and AST ($57.9. \pm 17.27$ IU/L) in free range tigers (Table1)) are comparatively higher with the values of BUN (23.4 ± 0.70 mg/dl), and AST (26.5 ± 4.7 IU/L) as recorded by Seal et al. (1987). The higher values in free range tigers might be associated with beasts of prey, its variety and intake of flesh in natural habits and habitat while zoo tigers are locally dependent on monitored diet in captivity.

Comprehensive information on haemato-biochemical parameters of free range tigers would be helpful for health monitoring and assessment of health status and prognosis of Bengal Tigers (*Panthera tigris tigris*) during treatment.

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