JOURNAL OF VETERINARY AND APPLIED SCIENCES
2015 VOL. 5(1): 7 - 15
Manuscript No. JVAS/2014/040; Received: 21/10/2014; Accepted: 10/03/2015
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

EFFECTS OF SPLENECTOMY ON THE HAEMOGRAM AND ERYTHROCYTE MORPHOLOGY OF NIGERIAN MONGREL DOG

Abubakar M. Mayaki[1*], Raphael O. C. Kene[2], Abubakar S. Yakubu[2]

[1]Department of Veterinary Physiology and Biochemistry, [2]Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University, Sokoto, Nigeria.

==========

ABSTRACT

The effects of splenectomy on haematology were evaluated by observing changes in haemogram and erythrocyte morphology in five splenectomized dogs from day 2 to day 30 post splenectomy. Splenectomy produced significant ($p < 0.05$) decreases in haematocrit, haemoglobin concentration, mean corpuscular haemoglobin concentration and red blood cell distribution width. The white blood cell and platelet counts counts increased significantly ($p < 0.05$) with platelet count being the more significantly ($p < 0.05$) affected on the 9^{th} and 16^{th} days post splenectomy whereas mean platelet volume decreased significantly on the 16^{th} day posts plenectomy. Granulocytes and lymphocytes were also significantly ($p < 0.05$) increased on the 2^{nd} and 9^{th} days respectively post splenectomy. However, no significant ($p > 0.05$) changes were seen in monocyte counts, platelet crit and platelet distribution width. Erythrocyte morphological abnormalities observed included increase in the circulation of crenated red blood cells, mild anisocytosis and poikilocytosis, which was characterized by presence of acanthocytes, codocytes, echinocytes and Howell-Jolly bodies. It was concluded that changes in haemogram of mongrel the dog following splenectomy were temporary as the blood parameters returned to normal while changes observed in erythrocyte morphology were consistent during the study period.

***Keywords*:** Nigerian dog, erythrocyte morphology, haemogram, splenectomy.

==========

INTRODUCTION

The spleen is the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes which initiates immune responses to blood-borne antigens [1, 2]. In mammals, the spleen has three main functions. Firstly, it elicits specific T or B lymphocyte-mediated immune reactions against antigens carried by the blood. Secondly, it aids in removal of materials that can be phagocytosed by splenic red pulp macrophages, including aged or abnormal red blood cells, microorganisms and leucocytes covered with immune complexes, And thirdly, it serves as a reservoir of erythrocytes, which are transfused into the circulation on sympathetic stimulation [3, 4].

***Correspondence: E-mail: bubakar241@yahoo.com; Tel:** +2348035233598
ISSN: 2315 - 6856

The dog's spleen, in contrast to that of humans , serves as an important reservoir of blood because it normally contains approximately twice the concentration of red cells as the peripheral blood [5]. Contraction of the spleen in a cardiovascular emergency and upon excitement or muscular activity may be life saving because highly concentrated splenic blood is expelled into the systemic circulation [5,6]. The slowing of passage of erythrocytes through the spleen subjects them to a specialized action the "pitting function" [7]. This concept visualizes removal of particles such as iron, Howell-Jelly bodies, nuclei and Heinz bodies from erythrocytes with the possible release of the "cleaned" cell to the circulation. Blood platelets are also concentrated in the spleen to the extent that one third of the total available platelets are retained for dynamic exchange with circulating platelets [8].

Despite the functions carried out by the spleen, splenectomized animal still live a normal life, as splenic functions can be assumed by the liver, lymph nodes and bone marrow [7]. Splenectomy had been the standard treatment for a number of splenic disorders such as splenic tumors, hereditary sperocytosis, splenic rupture and others [9,10,11]. Animals and man that had been subjected to total splenectomy showed marked alteration in their blood picture as well as serum proteins [9]. Waldmann *et al.* [10] reported that splenectomy of dog resulted in a significant reduction in circulatory erythrocytes due to reduced erythrocyte production. In a proportion of subjects, irregularly contracted or crenated, acanthocytic forms are also a feature, occasionally isolated erythroblasts are seen [11]. A transient or persistent thrombocytosis may occur post - splenectomy in humans [12], however this is not a consistent finding in dogs [13]. Rodents and dogs have been used to study the effects of splenectomy particularly on individual blood cells indices. The objectives of this study were to eval;uate the effects of splenectomy on the haemogram and erythrocyte morphology of Nigerian Mongrel dog.

MATERIALS AND METHODS

Experimental Animals

Five (5) mongrel dogs were used for the study. The ages of the dogs ranged from 8 to 12 months (10 ± 1.58 months) while their body weight was between 10 and 18 kg (15 ± 3.0 kg). The dogs were conditioned in the kennels of the Veterinary Teaching Hospital, Usmanu Danfodio University, Sokoto, for a month. Adequate food was given and water was sprovided *ad libitum*. They were also assessed to be apparently healthy on the basis of the result of hematological analysis, negative faecal flotation for helminthe ova and protozoan oocysts, and negative for haemoparasites.

Experimental procedure

Food and water were withheld from the dogs 12 hours before the surgery. The dogs were premedicated with 0.02mg/kg atropine sulphate and 1mg/kg xylazine 2%. Intravenous anesthesia was induced and maintained with 12mg/kg sodium pentobarbitone 6%. The splenectomy was performed as described by Archibald, [14]. Post splenectomy, the dogs were placed on prophylactic antibiotics, procaine penicillin and streptomycin at the dose rate of 20,000 IU and 10mg/kg respectively.

Three milliliters (3 ml) of blood was collected from the dogs by cephalic venipuncture prior to the splenectomy and also on the 2^{nd}, 9^{th}, 16^{th} and 30^{th} days post splenectomy for evaluation of haemogram and examination of erythrocyte morphology. The blood samples were collected into sample bottles containing ethylene diamine tetraacetic acid as anticoagulant.

The haematological indices evaluated were haematocrit (Hct), haemoglobin concentration (Hgb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW); total leucocyte count (WBC), and and differential leucocyte: platelet count (PLT), platelet crit (Pct), mean platelet volume (MPV) and platelet distribution width (PDW). All these haematological indices were evaluated using BC-2800 Vet Autohaematology analyzer.

Microscopic examination of thin films prepared with blood samples and stained with Leishman stain was used to evaluate the erythrocyte morphology according to the methods described by Linne *et al.* [15] and Monica [16]. The thin blood films were then observed under X100 (oil immersion) objective. The experiment lasted for 30 days.

Data analysis

Pre-splenectomy data were compared with post-splenectomy values using a one way analysis of variance, and variant means were separated post-hoc using the Turkey test (Graphpad Instat version 3.0). Significance was accepted at 5% probability level ($p < 0.05$. Results were presented as means ± standard deviations.

RESULTS

Erythrocyte indices

The effects of splenectomy on RBC, Hct, Hgb, MVC, MCH, MCHC and RDW were as shown in Table 1. There was no significant ($p > 0.05$) decrease in the RBC count post-splenectomy when compared to the pre-splenectomy values, although the mean values from day two post-splenectomy was lower than the day 0 values. A decrease in Hct below the pre-splenectomy value was recorded on day 2, 9 and 16 but significant ($p < 0.05$) decrease was observed only on the 9th day. The haemoglobin concentration was significantly ($p < 0.05$) decreased on the 2nd, 9th and 16th days post-splenectomy. There were no significant ($p > 0.05$) changes in the MCV and MCH when post-splenectomy values were compared to pre-splenectomy results, but the MCHC significantly ($p < 0.05$) decreased on the 2nd day post-plenectomy. The red blood cell distribution width was significantly ($p < 0.05$) decreased on the 9th day post-splenectomy. In spite of the fact that noticeable changes did occur post-splenectomy, the mean values of all the erythrocyte indices fell within the normal reference ranges on the 30th day of the experiment.

Table 1. Erythrocyte indices of Nigerian mongrel dogs before and after splenectomy.

	Days post splenectomy				
Parameters	0	2nd	9th	16th	30th
RBC x(10^{12}/L)	6.78 ±1.06^{a}	5.45±0.93^{a}	5.50±0.43^{a}	5.55±0.47^{a}	6.34±0.49^{a}
Hct (%)	43.6±6.39^{a}	35.8±5.77^{a}	34.4±3.86^{b}	35.8±2.66^{a}	41.7±2.38^{a}
Hgb g/L	140.8±22.03^{a}	106.6±18.58^{b}	108.0±8.37^{b}	111.2±7.79^{b}	127±7.58^{a}
MCV fL	64.4±1.58^{a}	65.96±2.70^{a}	62.56±2.52^{a}	64.60±1.58^{a}	66.20±7.34^{a}
MCH pg	20.74±0.40^{a}	19.54±0.21^{a}	19.64±0.11^{a}	20.04±0.31^{a}	20.18±2.28^{a}
MCHC g/L	322.2±4.09^{a}	297.0±14.75^{b}	314.6±13.98^{a}	310.6±5.77^{a}	304.6±2.30^{a}
RDW (%)	15.6±0.37^{a}	14.6±1.16^{a}	14.0±0.89^{b}	14.98±0.31^{a}	15.68±0.38^{a}

Mean ± SD on the same row with different superscripts are significantly different ($P < 0.05$)

Total and differential white blood cells

Table 2 showed the effects of splenectomy on total and differential white blood cells count in the dogs. There were increases in the values of total WBC on days 2 [21.16±6.85] and 9 [17.68±2.73)] above the pre-splenectomy value (6.0-17.0), with the value recorded on day 2 being significantly ($p < 0.05$) higher than the day 6 value. Absolute granulocyte count was significantly ($p < 0.05$) increased on the 2nd day post-splenectomy with value of 16.32±5.48 and absolute lymphocyte counts were also increased

significantly ($p < 0.05$) on the 9th and 30th days post-splenectomy with values of 5.54±1.40 and 5.34±1.17, respectively when compared to pre-splenectomy values. There were no significant ($p > 0.05$) changes in the absolute monocyte counts of the splenectomized dogs. Although changes occurred in the WBC and differential white blood count, but on the 30th day of the experiment, all the mean values were within the reference ranges except for the lymphocyte count.

Table 2. Total and differential white blood cell counts before and after splenectomy in Nigerian mongrel dogs

	Days post splenectomy				
Parameters	0	2nd	9th	16th	30th
WBC x 10^9/L	12.18±0.85[a]	21.16±6.85[b]	17.68±2.73[a]	15.18±3.85[a]	10.80±1.44[a]
Gran x10^9/L	8.88±1.99[a]	16.32±5.48[b]	10.88±1.65[a]	10.76±1.65[a]	4.84±0.57[a]
Gran (%)	72.48±13.19[a]	76.38±4.34[a]	61.66±4.60[a]	72.28±7.19[a]	45.22±6.05[b]
Lymp x10^9/L	2.62±1.33[a]	3.90±1.34[a]	5.54±1.40[b]	3.46±1.79[a]	5.34±1.17[b]
Lymp (%)	21.86±11.96[a]	18.76±2.92[a]	31.04±4.44[a]	21.68±5.62[a]	49.20±6.33[b]
Mono x10^9/L	0.68±0.15[a]	0.94±0.27[a]	1.26±0.11[a]	0.96±0.56[a]	0.62±0.28[a]
Mono (%)	5.66±1.43[a]	4.86±1.54[a]	7.28±0.82[a]	6.06±1.85[a]	5.62±2.04[a]

Mean ± SD on the same row with different superscripts are significantly different ($P < 0.05$)

Platelet indices

The effects of splenectomy on platelet indices are shown on Table 3. There were significant ($p < 0.05$) increases in the value of PLT on the 9th [812±118.6] and 16th [821.4±110.8] days post-splenectomy. The MPV was significantly ($p < 0.05$) decreased on the 16th [6.4(±0.43)] day post-splenectomy when compared to the pre-splenectomy value [7.68±1.07]. No significant ($p > 0.05$) changes were observed in the Pct and PDW of the splenectomized dogs, although increase in Pct above the normal reference range (0.13 – 0.40) was observed on day 9 [0.42±0.24] and16 [0.53±0.07]. On the 30th day of the experiment, the mean values of all the platelets indices were within normal the reference ranges.

Table 3. Platelets indices of Nigerian mongrel dogs before and after splenectomy

	Days post splenectomy				
Parameters	0	2nd	9th	16th	30th
PLT x10^9/L	244.4± 81.8[a]	345.8± 64.5[a]	812.2± 118.6[b]	821.4± 110.8[b]	230.0± 14.5[a]
Pct (%)	0.36±0.34[a]	0.27± 0.05[a]	0.42± 0.24[a]	0.53± 0.07[a]	0.19±0.005[a]
MPV fL	7.68± 1.07[a]	7.92±0.48[a]	7.04± 0.38[a]	6.40±0.43[b]	8.30±0.34[a]
PDW	15.54± 0.84[a]	15.96±0.23[a]	15.84±0.34[a]	15.24±0.23[a]	15.26±0.20[a]

Erythrocyte morphology

The blood pictures obtained from blood films 2 to 30 days after splenectomy showed characteristic morphological changes of erythrocytes particularly poikilocytosis, mild anisocytosis and crenated erythrocytes. The poikilocytosis was characterized by presence in blood film of echinocytes, ovaloechinocytes, acanthocytes, codocytes, schistocytes and Howell-Jolly bodies. The crenated red cells and mild anisocytosis were observed on day 2 (Figure 1) while on day 9, echinocytes and ovaloechinocytes are more in circulation (Figure 2). Days 16 and 30 were characterized by presence of acanthocytes, codocytes and schistocytes in blood film (Figures 3 and 4).

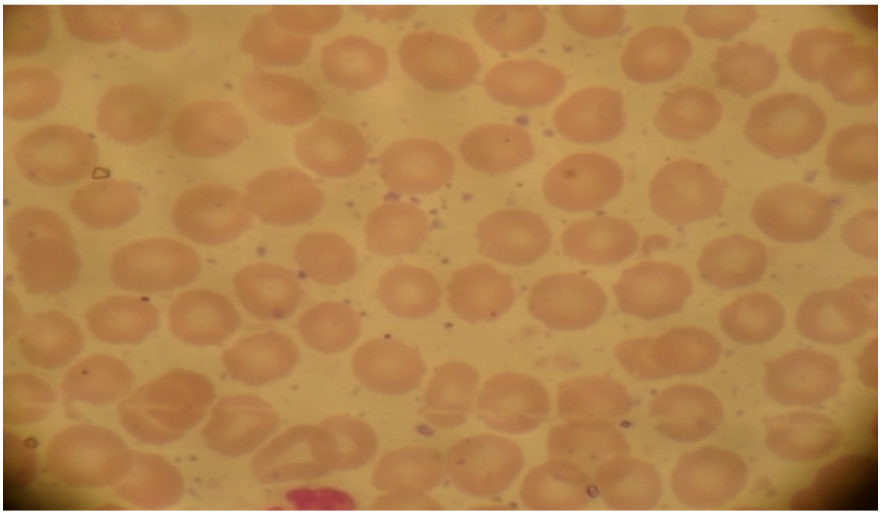

Figure 1. Mild anisocytosis with crenated red cells in the blood smear of spenectomized dog

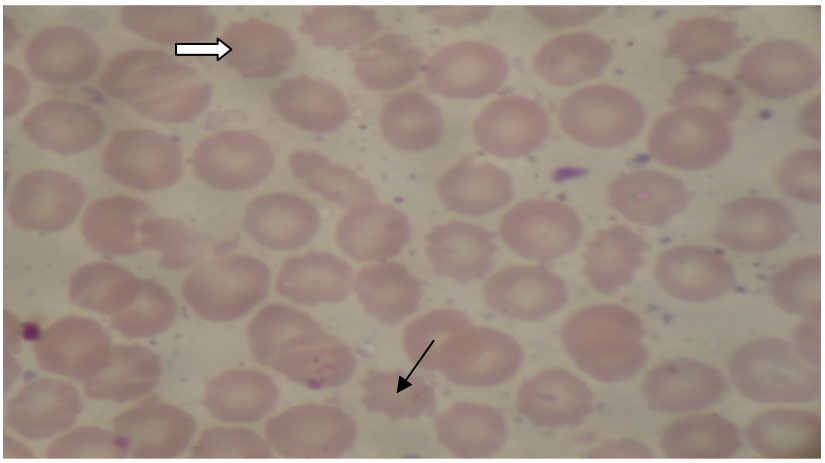

Figure 2. Echinocytes (big arrow) and ovaloechinocytes (thin arrow) in the blood smear of spenectomized dog

DISCUSSION

The results of this study showed clearly that characteristic changes occur in the haemogram following splenectomy. The decrease in Hct and Hgb values were consistent decreases in RBC values particularly on days 2, 9 and 16 post-splenectomy reflecting anaemia due to blood loss during the surgery. Significant decrease in MCHC was also observed on the 2nd day post-splenectomy. Significant decreases in

erythrocyte values after total splenectomy have been documented by previous research in mice [17], dogs [18], pig [19] and humans [20]. The post-operative recovery time for erythrocytes count in this experiment was recorded to be 30 days. This is in agreement with the reports of Esmart *et al*. [21] who stated that the mean total erythrocytes count showed slight decrease after partial splenectomy and then increased to reach almost the original levels at one month after the operation. Lober [18] reported that it took 2 to 3 weeks for dog to regenerate its normal range. This change in erythrocytes indices, particularly the reduction in circulatory erythrocytes, could be due to decrease in haematopoiesis [10] and or blood loss during the surgery . The decrease in Hgb and MCHC may be as a result of inadequate supply of iron due to haemorrhage or blood loss, for the developing erythroblasts and haemoglobin synthesis. The RDW usually refelected variations in the size of circulating erythrocytes (anisocytosis). Bessman *et al.* [22] reported that changes in RDW may be due to lack of iron, vitamin B_{12} or folic acid, but its normal in

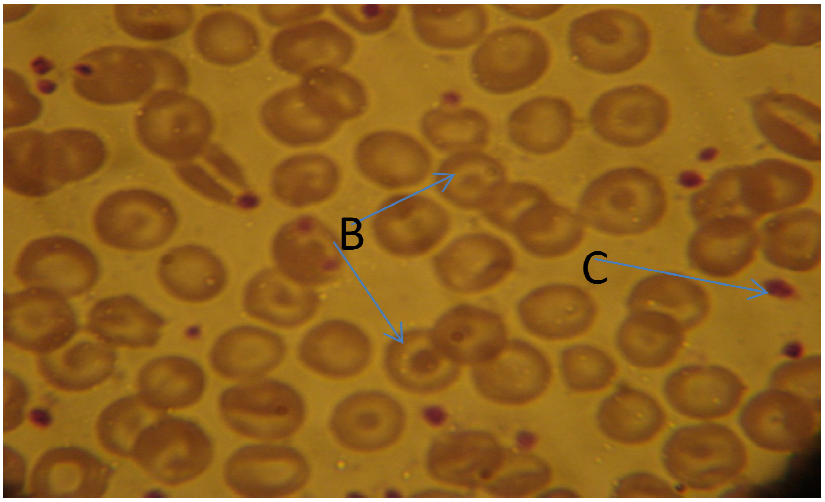

Figure 3. Codocytes (B) and larger platelet (C) in the blood smear of spenectomized dog

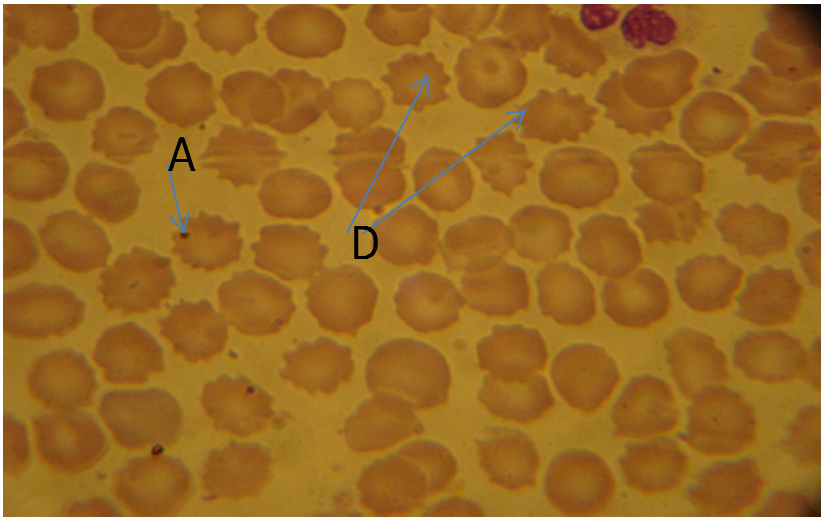

Figure 4. Acanthocytes(D) and howell-jolly body(A) in the blood smear of spenectomized dog

defects such as hypoplastic anaemia. Significant decrease of RDW was observed on day 9 during the experiment. Therefore, the variation in RDW value recorded may as well be due to decrease in iron availability following blood loss during surgery or as a result of different sizes of circulating erythrocyte seen in the blood smear reflecting loss of splenic 'pitting function' following splenectomy.

The immediate leucocytosis observed in the first nine days of the experiment could probably be due to the stress associated with the surgery and /or due to increase in inflammatory reaction in response to healing or haemorrhage during the surgery [19,20]. The leucocytosis recorded in the present study was consistent with findings in some previous studies [23, 24]. Hoffbrand *et al*. [11] reported that neutrophilic leucocytosis was the immediate response in majority of human subjects which was later replaced by a significant and permanent increase in both lymphocytes and monocytes, although in these studies, the immediate leucocytosis observed was later replaced by significant lymphocytosis as observed on the 9^{th} and the last day of the experiment but no changes was observed in the monocytes counts. Increases in total leucocyte counts post-splenectomy and persistent leucocytosis were documented in mice [25], rabbits [26] and humans [27]. Spika *et al*. [17] reported a significant increase in neutrophil count and the lymphocytes count remained unchanged while Bessler *et al*. [25] recorded significantly higher lymphocytes count but neutrophil count remained unchanged. The immediate leucocytosis observed post-splenectomy in this study is not regarded as a specific change for splenectomy but could be as a result of inflammatory response to surgical trauma.

The significant increase in PLT on the 9^{th} and 16^{th} day postsplenectomy suggest a reactive thrombocytosis which was transient as all the platelet indices were within the normal reference values on the 30^{th} day of the experiment. This result showed that spleen plays a major role in platelet regulation, as it is the primary site of destruction of platelets, which is why thrombocytosis is seen with hyposplenism [28]. It has been documented that reactive thrombocytosis is a predictable finding after splenctomy with the platelet count peaking at 1 to 3 weeks and returning to normal in weeks, months and rarely years [29]. The result of the present study showed that platelet count peaking at 16^{th} day and returning to normal in a month. The thrombocytosis recorded following splenectomy may be due to absence of the spleen which plays a major role in the removal of circulating platelets.
The blood picture shown in figures 1,2,3 and 4 obtained on the blood films showed that spleen exercises a level of control over erythropoiesis and thus erythrocyte morphology. In the absence of the spleen the dog erythrocyte morphology changes due to erythrocytes fragmentation which led to the poikilocytosis, or oxidative injury of the erythrocytes [7]. The absence of the spleen following splenectomy leads to less of sequestration role of the spleen such that erythrocyte with inclusion, fragmented erythrocytes like echinoctes, codocytes, acanthocytes will be in circulation.

CONCLUSION

Based on the results of this study, it was concluded that the haematological changes following splenectomy are transitory and not permanent. However, erythrocyte morphological changes such as poikilocytosis, particularly presence of codocytes, echinocytes, acanthocytes, Howell-jolly bodies observed were consistent in the splenectomized dog during the study period.

REFERENCES

1. Nolte, M. A., Hamann, A., Kraal, G. and Mebius, R. E. (2002). The strict regulation of lymphocyte migration to splenic white pulp does not involve common homing receptors. *Immunology,* 106 (3): 299 – 307. DOI: 10.1046/j.1365-2567.2002.01443.x
2. Balogh, P., Horvath, G. and Szakal, A. K. (2004). Immunoarchitecture of distinct reticular fibroblastic domains in the white pulp of mouse spleen. *Journal of histochem cytochem,* 52 (10) : 1287 – 1298. DOI: 10.1177/002215540405201005
3. Steiniger, B. and Peter, B. (2000). Microanatomy and function of the spleen. *Advances in Anatomy, Embryology and Cell Biology,* 151: 1 – 101. ISBN:978-3-642-570088-9

4. Mark, F. C. (2006). Normal structure, function and histology of the spleen. *Toxicologic Pathology*, 34(5):455 - 465. DOI: 10.1080/01926230600867743
5. Schalm, O. W. (2010). Schalm's Veterinary Haematology. 6th Edition, Weiss, D. J. and Wardrop, K. J. (Eds). Wiley-blackwell Ltd. pp 1232. ISBN- 13:978-0-8138-XXXX-X/2007
6. Hartwig, H. and Hartwig, H. G. (1985). Structural characteristics of the mammalian spleen indicating storage and release of red blood cells. Aspects of evolutionary and environmental demands. *Experientia*, 41(2): 159 - 163.
7. Crosby, W. H. (1959). Normal functions of the Spleen relative to red blood cells. *Blood*. 14. 339.
8. Aster, R. H. (1966). Pooling of platelets in the spleen. Role in the pathogenesis of 'hypersplenic' thrombocytopenia *Journal of Clinical Investigations*, 45: 645.
9. Anosa, V. O., Isoun, T. T. and Oladosu, L. A. (1979). Splenectomy in sheep; technique,haematological changes and the haematology of the precipitated anaplasmosis and babesiosis. *Veterinary Medicine, A*. 26: 327 – 336.
10. Waldmann, T. A., WeissMan, S. M. and Berlin, N. (1960). The effect of splenectomy on erythropoesis in the Dog. *Blood*, 15: 873.
11. Hoffbrand, A. V., Catovsky, D. and Tuddenham, E. G. D. (2005). *Postgraduate Haematology* Volume 1, 15th Edition. Blackwell Publishing Ltd. Pp. 358. ISBN-13:978-1405108218.
12. Schafer, A. I. (2006). Essential thrombocythemia and thrombocytosis . In: *Williams Hematology*, 7th Edn., Lichtman, M. A., Beutler, E., Kipps, T. J. (Eds). New York : McGraw - Hill , pp. 1785 – 1794. ISBN-13:978-0071435918.
13. Richardson, E. F. and Brown, N. O. (1996). Hematological and biochemical changes and results of aerobic bacteriological culturing in dogs undergoing splenectomy. *J Am Anim Hosp Assoc* 32(3): 199 – 210.
14. Archibald, J. (1972). *Canine Surgery*. 2nd Edition. American Veterinary Publications Inc. USA, Pp. 808.
15. Linne, J. J. and Ringsrud, K. M. (1979). *Basic Techniques for the Medical Laboratory*. 2nd Edition. Mc Graw-hill. Pp. 256.
16. Monica, C. (2000). *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press. pp. 332 - 333.
17. Sipka, S. Jr., Brath, E., Tòth, F. F., Fabian, A., Krizsan, C., Barath, S., Sipka, S., Nemeth, N., Balint, A., Furka, I. and Miko, I. (2006). Distribution of peripheral blood cells in mice after splenectomy or autotransplantation. *Microsurgery*, 26(1): 43 - 49. ISSN 0033-8419.
18. Lorber, M. (1958). The Effects of Splenectomy on the Red Blood Cells of the Dog with Particular Emphasis on the Reticulocyte Response. *Blood*, 13 (10): 972 - 985. ISSN 0006-4971
19. Nina, P-M., Anja, V., Josipa, M., Drazen, V. and Matko, K. (2012). The Effects of Splenectomy and Autologous Spleen Transplantation on Complete Blood Count and Cell Morphology in a Porcine Model. In: *Hematology - Science and Practice*.Lawrie, C. (Ed.), Pp 553 – 572. ISBN: 978-953-51-0174-1.
20. Knezevic, S., Stefanovic, D., Petrovic, M., Djordjevic, Z., Matic, S., Artiko, V., Milovanovic, A. and Popovic, M. (2002). Autotransplantation of the spleen. *Acta Chirurgica Jugoslavica*, 49 (3): 101 - 106. ISSN 0354-950X
21. Esmart, M. E., Abbas, M., Kishta, S., and Sheta, E. M. E. (2001). Partial Splenectomy using reloadable linear staples in dogs. *Egyptian Journal of Surgery*. 2 (3): 616 – 623.
22. Bessman, J. D., Gilmer, P. R. and Gardner, F. H. (1983). Improved classification of anemias by MCV and RDW. *American Journal of Clinical Pathology*, 80 (3): 322 – 326. ISSN: 0002-9173.
23. Pister, P. W. and Pachter, H. L. (1994). Autologus Splenic transplantation for Splenic trauma. *Annals of Surgery*, 219 (3): 225 – 235.
24. Toutouzas, K. G., Velmalous, G. C., Kaminski, A., Chan, L. and Demetriades, D. (2002). Leukocytosis after posttraumatic splenectomy: a physiologic event or sign of sepsis? *Archives of Surgery*, 137: 924 – 929.

25. Bessler, H., Bergman, M., Salman, H., Beilin, B. and Djaldetti, M. (2004). The relationship between partial splenectomy and peripheral leukocyte count. *Journal of Surgical Research*, 122 (1): 49 – 53. ISSN 0022-4804
26. Karagulle, E., Hoscoskun, Z., Kutlu, A. K., Kaya, M. and Baydar, S. (2007). The effectiveness of splenic autotransplantation: an external study. *Turkish Journal of Trauma and Emergency Surgery*, 13 (1): 13 - 19. ISSN 1306-696
27. Zhang, H., Chen, J., Kaiser, G. M., Mapudengo, O., Zhang, J., Exton, M. S. and Song, E. (2002). The Value of Partial Splenic Autotransplantation in Patients with Portal Hypertension. *Archives of Surgery*, 137 (1): 89 – 93. ISSN 0004- 0010
28. Khan, P. N., Nair, R. J., Olivares, J., Tingle, L. E. and Li, Z. (2009). Postsplenectomy reactive thombocytosis. *Baylor University Medical Center Proceedings*, 22 (1): 9 - 12.
29. Shirley, P. L. (2004). Thrombocytosis. In. *Wintrobe's Clinical Hematology*. 11th Edn., Vol. 2. Greer, J. P., Foerster, J., Lukens, J. N., Rodgers, G. M., Paraskevas, F. and Glader, B. E. (Eds.). Lippincott Williams and Wilkins, Philadelphia.