

AN ANNUAL STUDY OF STOMATOCYTES : A RED CELL ANOMALY IN THE MALE AND FEMALE FALSE VAMPIRE BAT *Megaderma lyra lyra* (Geoffroy)

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ABSTRACT

The presence of red blood cell abnormalities can give important diagnostic clues for the well being of any species. It is important therefore, that all features of the erythrocytes be assessed including number, color, size, polychromasia, the presence of precursors, alterations in shape, inclusions, the presence of RBC parasites and arrangement. Erythrocytes can occur in many different shapes. Most of them are pathological and can be involved in diseases such as hemolytic anemias and sickle cell anemia. Stomatocytes are such types of red cells in the stained film have a linear area of pallor in the centre imparting the cell an appearance of mouth-like opening. The recorded data of stomatocytes throughout the year was in the range of 4-15% from both the sexes pointing to mild stomatocytosis. Aim of the present study points to mild stomatocytosis with similar factors for their transformation as described in the literature such a condition may mildly obstruct the peripheral circulation causing less pathogenecity.

Key Words : Haematology, Microchiroptera, Bat, *Megaderma lyra lyra*, Blood, Stomatocytes

INTRODUCTION

The human red blood cell is characterized by its discoid shape and its ability to undergo extensive passive deformation during repeated passage through the narrow capillaries of the microvasculature during its 120-day life span in the circulation.¹ A hereditary defect in human red blood cells associated with stomatocytosis and haemolytic anemia described as stomatocytosis to describe red cells with a well demarcated linear unstained area across their centre suggesting a mouth-like orifice in the cell. Since then stomatocytosis has been observed both as an acquired and as a congenital condition and now it is clear that congenital stomatocytosis is a heterogeneous group of disorders, some associated with disturbances in red cell electrolyte concentration and some not. Erythrocytes can occur in many different shapes. Most of them are pathological and can be involved in diseases such as hemolytic anemias and sickle cell anemia. Only three kinds of red blood cells are not pathological. Echinocytes,

stomatocytes and discocytes can occur in blood stream of healthy organism. The echinocyte-discocyte-stomatocyte transformation protects red blood cells from lysis caused by stomatocytogenic agents such as hypotonic-saline, acidic pH, cationic amphiphiles etc.² Red cell shape is encoded in the mechanical properties of the membrane. The plasma membrane contributes bending rigidity, the protein-based membrane skeleton contributes stretch and shear elasticity. When both effects are included, membrane mechanics can reproduce in detail the full stomatocyte-discocyte-echinocyte sequence.³

AIMS AND OBJECTIVES

To describe the presence of stomatocytes in the annual collection of blood samples of *Megaderma lyra lyra* which resembles some human disorders in which stomatocytosis of the red cells is a characteristic feature, which is associated with glutathione deficiency and disordered red cell cation and water content.

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MATERIAL AND METHODS

Collection of specimens

The specimens of *Megaderma lyra lyra* were collected with the help of a mist net placed at the entrance of the underground mines at Mansar / Kandri near Nagpur, Maharashtra (20°92"N 78°95"E), once every calendar month throughout the complete reproductive cycle from April 2009-March 2010.

Blood sampling

The bats were held in hands and no anesthesia was used at the time of sample collection. 2 ml of blood was collected into sterile eppendorf tubes with no anticoagulants (neither EDTA nor heparin) after puncturing a wing vein. After blood sampling each bat was released.

Preparation of a blood film

Blood from males and females were used

without any anticoagulant directly from the syringe or needle immediately after collection by applying the wedge smear or the slide and coverglass technique. A routine preparation of at least three smears per blood sampling were performed. The air-dried smears were kept in a dust free environment until they were stained.

Giemsa staining of blood smeared slide

The occurrence of stomatocytes in red blood cells were studied throughout the reproductive cycle, from April 2009 to March 2010 by staining blood smears with Giemsa for 24 hours.

Statistical analysis

The data was analyzed statistically, standard errors were calculated on the basis of which graphs were plotted to compare the seasonal variation.⁴ **Table 1** and **Fig. 1** shows

Table 1 : The results of hematologic investigations

Specimen (n = 3)	Date of collection	Time of collection	Body weight(g)	Occurrence of Stomatocytes (%)
Female	22/4/2009	3.00 p.m.	39	Occurrence (8%)
Male			30	Occurrence (13%)
Female	19/5/2009	2.30 p.m.	36	Non occurrence
Male			32	Occurrence (9%)
Female	25/06/2009	10.30 a.m.	34	Non occurrence
Male			31	Occurrence (15%)
Female	30/07/2009	11.00 a.m.	34	Non occurrence
Male			32	Occurrence (4%)
Female	16/08/2009	10.30 a.m.	34.5	Non occurrence
Male			31	Occurrence (12%)
Female	02/09/2009	3.30 p.m.	33	Non occurrence
Male			32	Non occurrence
Female	04/10/2009	11.25 a.m.	33	Occurrence (11%)
Male			32	Non occurrence
Female	05/11/2009	11.30 a.m.	34	Non occurrence
Male			31.5	Non occurrence
Female	08/12/2009	11.00 a.m.	33.5	Occurrence (10%)
Male			31.5	Non occurrence
Female	07/01/2010	11.30 a.m.	34	Occurrence (13%)
Male			30	Non occurrence
Female	02/02/2010	2.00 p.m.	34	Non occurrence
Male			32.5	Occurrence (9%)
Female	03/03/2010	11.00 a.m.	36	Occurrence (10%)
Male			32	Non occurrence

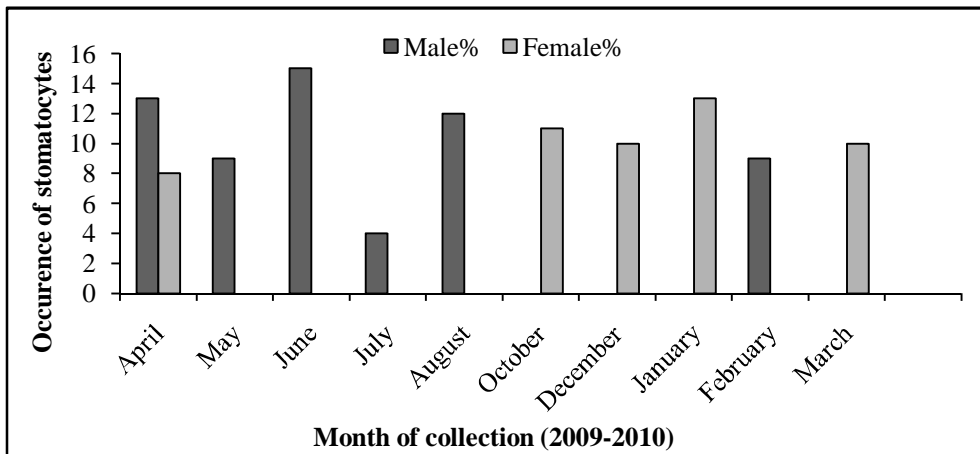


Fig. 1 : Graphical representation of stomatocytes in red blood cells from April 2009 to March 2010

pertinant data regarding collection of *Megaderma lya lya* used for the present study is also been incorporated. The number in paranthesis is for animals used.

RESULTS AND DISCUSSION

Stomatocytes were seen during the months

April (8%),October (11%),December (10%), January (13%) and March (10%) from females (**Table 1, Fig. 2(a) and Fig. 2(b)**) and during April (13%), May (9%), June (15%), July (4%), August (12%) and February (9%) from males (**Table 1, Fig. 3(c) and Fig. 3(b)**) describing similarity in both the sexes.

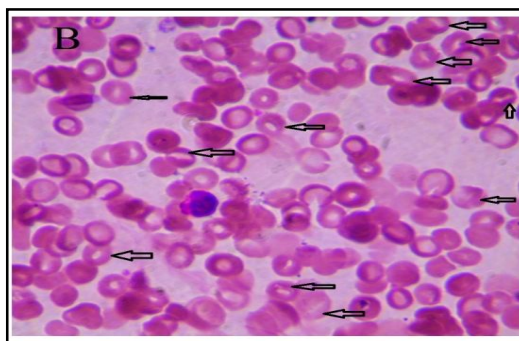
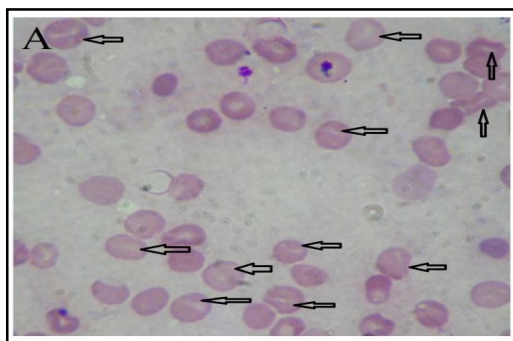


Fig. 2 : Stomatocytes were observed in the blood smears in panels A and B from females collected on 22/04/2009 and 04/10/2009 respectively (arrow). The stomatocytes have a linear area of pallor in the centre which gives the cell in appearance of mouth like opening (arrow)

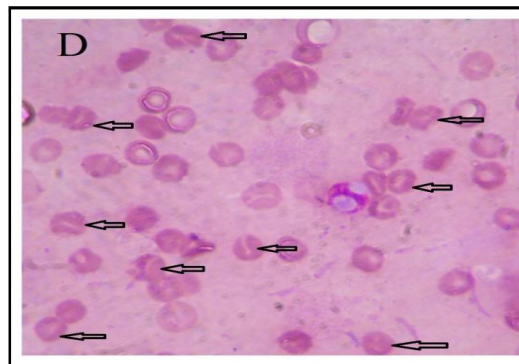
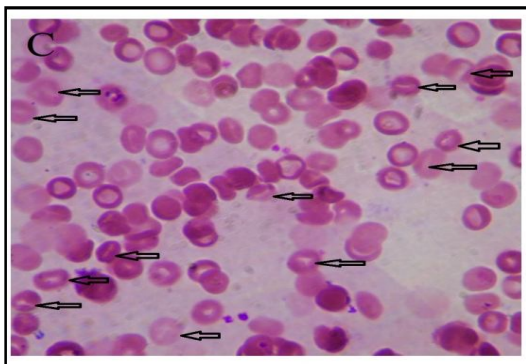


Fig. 3 : Stomatocytes were observed in the blood smears in panels C and D from males collected on 19/05/2009 and 02/02/2010 respectively (arrow). The uniconcave red blood cells, stomatocytes with a slit-like area of central pallor

The annual study of blood smear of the false vampire bat *Megaderma lyra lyra* revealed that the occurrence of stomatocytes from both the sexes were in the range of 4-15%. On comparison with the literature in human revealed that the pathological condition of stomatocytosis occur only when stomatocytes are in the range of 30-40%.^{2,3} The stomatocytes are overhydrated cells, which appear as an intermediate form in the transformation of echinocytes to diskocytes and then to stomatocytes. Thus the low range of stomatocytes explains non-pathogenicity in *Megaderma lyra lyra* and are similar to the observation⁵ for the transformation of normal red blood cells into stomatocytes there are a number of reasons given in the literature which are applicable to our present studies. Some of the causes for stomatocytosis are an increased permeability of the red cell membrane for monovalent cations.^{6,7} However, are of the opinion that stomatocytosis may be caused by loss of membrane surface area rather than by the increased cation uptake or oxidative degradation of membrane lipids and alteration of internal microviscosity.⁸⁻¹²

CONCLUSION

Red Blood Cells (RBCs) membrane and cytoplasmic structural damage consequently alters RBCs rheologic properties: an alteration of the RBCs discoid shape to stomatocytes, a diminution of erythrocyte deformability, an enhancement of osmotic fragility and cell aggregability which effects impaired blood fluid behaviour and thus contribute to obstruct peripheral circulation and provides anemia. Similarly the erythrocyte membrane permeability to monovalent cations is associated with mutations in the Rh-associated glycoprotein gene or it may be associated with intrinsic structural defect of the RBC membrane or may be induced by abnormal composition of the plasma. Some have interpreted more or less similar reasons for stomatocytosis such as redistribution of bilayers lipid, modification of Donnan's equilibrium and interaction of band 3 protein with different type of external factors. Stomatocytosis may be due to glutathione deficiency. Ion transportation defect may be another reason for stomatocytosis.

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