JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Cell and Molecular Biology

Research Article

Comparative Morphometry of Erythrocytes of Different Fish Species

Mesut Yilmaz[1*], Olgaç Guven, Beria Falkali Mutaf[1]

[1]*Akdeniz University, Faculty of Aquatic Sciences and Fisheries, Antalya, Turkey*

[2]*Middle East Technical University, Institute of Marine Sciences, Erdemli-Mersin, Turkey*

**Corresponding author: Mesut Yilmaz, Middle East Technical University, Institute of Marine Sciences, Erdemli-Mersin, Turkey, Email: myilmaz@akdeniz.edu.tr*

Received: 07-31-2014

Accepted: 12-16-2014

Published: 02-24-2015

Abstract

The study was conducted to determine morphometric characteristics of red blood cells (RBCs) of different fish species caught during trawl operations carried out between 20 - 600 m depth range in Antalya Bay, Turkey. Cell and nuclear sizes of RBCs of 11 fish species examined and measured. Variations in RBCs overall shapes and sizes were observed among the species. While *S. solea, P. erytrinus, T. trachurus, U. moluccensis, M. barbatus, L. mormyrus, L. whiffiagonis* and *H. dactylopterus* erytrocytes maintained smooth morphology, sickling with a prominent hemoglobin bar appeared in *N. randalli, E. aeneus* and *M. merluccius*. The latter group of fishes seemed to manage to survive successfully at least 1 hour in controlled environment created on board even with haemoglobin polymerisation caused by capture stress. The present study showed that sickling shape in RBCs depends on the presence of solid cytoplasmic bar of hemoglobin that distorted cell shape. Moreover, statistically different size measurements were obtained between the normal and sickle cell groups of *E. aeneus* and *N. randalli* at the significance level of $P<0.001$. Nuclei, in the cells changing into sickle forms, showed shrinkage to compact structures, without any significant changes in NL/NW values. The mechanism of haemoglobin polymerisation in fishes, whether a permanent characteristics (physiological mechanism) or only appear subsequent to capture stress (adaptive mechanism), is a question to be solved in future studies.

Keywords: Red Blood Cell; Morphology; Fish; Sickling; Haemoglobin

Introduction

Fish red blood cells (RBCs) considered prototype of the circulating nucleated, hemoglobin-bearing cell that is phylogenetically retained by all other non-mammalian vertebrates. Fish erythrocyte is a permanently nucleated, hemoglobin-ladened, oval shaped, flattened, biconvex disc [1]. Its nuclear size and regular cell shape varies significantly among the species. Shape deformations, the most obvious of which are the sickle shape appear to be widespread in fishes [2]. It, however, was suggested to prone to hemoglobin oxygenation state [3]. High concentrations of Haemoglobins (Hbs) inside RBCs optimize O2 transport to the deep tissues.

Here we conduct this study to obtain size measurements in order to establish reference values for different 7 fish species missing and additional measurements for 4 species in the conventional list [4]. Also the presences of sickling of RBCs in sampled specimens and discuss the possible effects with capture stress evaluated.

Materials and Methods

This study was carried out in the Antalya Bay, Turkey. Two hauls were evaluated for recent study. Individuals were caught with bottom trawling and sampling was carried out between 20-100m depth range on continental shelf and towing duration was 1 and 2 hours, respectively. Trawl operations were conducted with R/V "Akdeniz SU" at an average speed of 2.5 nautical miles/h (between 2.2-2.7)

with a conventional bottom trawl. Randomly taken live caught specimens placed in a 500 L-polyester outdoor fish tank which were continuously surface water entrance (3-4 lt/min) using two electric water pumps. Blood samples were taken by heparinised-single-use sterile syringes from caudal vein of living fish. Smear slides were prepared immediately in the laboratory of R/V Akdeniz SU.

Blood samples for light microscopy were taken from different number of individuals were used for each of 11 species *(Lithognathus mormyrus, Solea solea, Mullus barbatus, Pagellus erytrinus, Trachurus trachurus, Lepidorhombus whiffiagonis, Nemipterus randalli, Epinephelus aeneus, Upeneus moluccensis, Helicolenus dactylopterus* and *Merluccius merluccius)*. Thin blood films were made on glass slides, fixed in ethanol immediately after sampling, air dried and subjected to May Grünwald-Giemsa staining. Three slides and 3 areas on each slide were examined from the individuals of each fish species. On each area length and width of different number of erytrocytes and their nuclei were measured under objective 40X lense on Olympus 41CX light microscope. For each sample at least 120-150 RBCs were studied and assigned as normal (N) and sickled (S). Actual measurements at µm level were done by ImageJ software [5-7]. All data are presented as means± s.d. Statistically significant differences between samples were accepted at P<0.001 and were tested by Student's t-test.

Results

The morphology of red blood cells (RBCs) were compared in 11 fish species and the values of morphometric parameters are shown in Table1.

Table1. Morphometric characteristics of the red blood cells of 11 fish species studied.

No	Species	Ns	Nc	EL µm (mean±sd)	EW µm (mean±sd)	EL/EW	NL µm (mean±sd)	NW µm (mean±sd)	NL/NW
1N	*Epinephelus aeneus (Normal Cell)*	5	150	10.5±0.9^a	6.3±0.6	1.650	4.7±0.5^a	3.3±0.4^a	1.441^a
1S	*Epinephelus aeneus (Sickled Cell)*	5	150	11.5±0.8^b	-	-	3.9±0.4^b	2.7±0.4^b	1.462^a
2N	*Nemipterus randalli (Normal Cell)*	5	150	8.6±0.6^a	6.0±0.5	1.435	4.7±0.6^a	3.0±0.5^a	1.548^a
2S	*Nemipterus randalli (Sickled Cell)*	5	150	8.9±0.6^b	-	-	3.4±0.3^b	2.3±0.2^b	1.495^a
3	*Upeneus moluccensis*	1	120	8.1±0.7	4.9±0.4	1.676	3.1±0.4	2.2±0.3	1.401
4	*Pagellus erythrinus*	2	120	9.2±0.6	5.0±0.6	1.857	3.4±0.3	2.3±0.3	1.450
5	*Mullus barbatus*	1	120	8.7±0.6	6.8±0.5	1.279	3.7±0.5	2.7±0.4	1.370
6	*Trachurus trachurus*	3	120	8.8±0.8	5.8±0.6	1.531	3.6±0.4	2.6±0.3	1.378
7	*Solea solea*	5	150	10.7±0.8	7.1±0.6	1.507	4.3±0.9	3.0±0.4	1.433
8S	*Merluccius merluccius (Sickled Cell)*	5	150	12.5±1.0	-	-	3.7±0.5	2.2±0.3	1.635
9	*Lithognathus mormyrus*	1	120	7.3±0.4	5.5±0.6	1.327	3.1±0.3	2.4±0.3	1.291
10	*Lepidorhombus whiffiagonis*	4	120	10.8±1.0	5.8±0.6	1.862	3.1±0.4	2.6±0.4	1.192
11	*Helicolenus dactylopterus*	4	120	10.7±0.8	7.0±0.5	1.529	3.9±0.7	2.6±0.3	1.500

Notes: Ns: Number of Specimen, Nc: Number of measured cell, EL: Erythrocyte Length, EW: Erythrocyte Width, NL: Nucleus Length, NW: Nucleus Width. Values, which are normal and sickle cell groups of *Epinephelus aeneus* (1N and 1S) and *Nemipterus randalli* (2N and 2S) in the same column, having different letters are significantly different (P<0.001).

Light microscopy showed that while the *S. solea, P. erytrinus, T. trachurus, U. moluccensis, M. barbatus, L. mormyrus, L. whiffiagonis* **and** *H. dactylopterus* cells maintained smooth morphology (Figure 1), the outline in RBCs of 3 studied species (*N. randalli, E. aeneus* and *M. merluccius*) changed dramatically and tended to show a distinct cytoplasmic bar. These cells were referred to sickle cells because of their appearance.

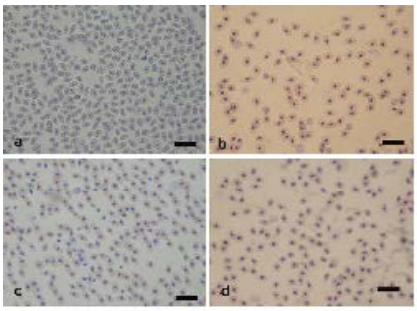

Figure 1. Smooth morphology of RBCs of *Upeneus moluccensis*(a), *Helicolenus dactylopterus*(b), *Lepidorhombus whiffiagonis* (c) and *Trachurus trachurus*(d). Scales show 20 µm

The study showed that sickling shape in RBCs depends on the presence of solid cytoplasmic bar of hemoglobin that distorted cell shape. Fraction of sickled-RBCs varied among these species even between individuals. *M. merluccius*, trawled from 100m depth, RBCs were totally sickled in shape (Figure 2) while sickled RBCs were observed in some individuals of *N. randalli* (Figure 3) in tandem blood sampling, but only few were seen in *E. aeneus* (Figure 4) blood smears, both were trawled from 20m depth like *S. solea, P. erytrinus, T. trachurus, U. moluccensis, M. barbatus* with normal RBCs.

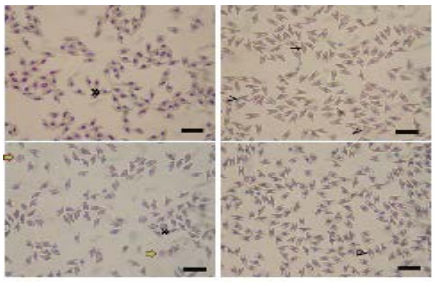

Figure 2. RBCs of *Merluccius merluccius*. Scales show 20 µm. Arrows indicate: Normal cell, Δ Angled cell, Compact nucleated cell, » Sickled cell.

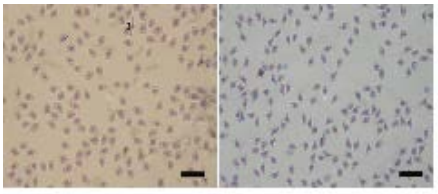

Figure 3. RBCs of *Nemipterus randalli*. Scales show 20 µm. Arrow indicates: › Bar shaped cell.

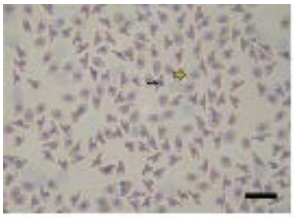

Figure 4. RBCs of *Epinephelus aeneus*. Scale shows 20 µm. Arrows indicate: Normal cell, Compact nucleated cell.

In some *M. merluccius* individuals RBCs showed a typical sickle shape with a single Hb bar, but some others with 2 bars in a triangular cell shape seemingly unconnected blood sampling order of the individuals (Figure 2). Statistically different size measurements were obtained between the normal and sickled cell groups of *E. aeneus* (1N and 1S) and *N. randalli* (2N and 2S) with the significance level of $P<0.001$. Nuclei, in the cells changing into sickle forms, showed shrinkage to compact structures, without any significant changes in NL/NW values (Table 1).

Discussion and Results

The ability of red cells to alter their shape reversibly is generally agreed to facilitate their circulation, conversely, non-deformable cell shape is observed in several groups of fishes [2,8].

Our results showed that the studied morphometric characters were comparable and close to those previously recorded values. *M. barbatus* and *Trachurus* sp. erytrocytes were measured smaller, while *Pagellus* sp. and *S. solea* within the size range of those in the literature [4].

Nemipterus randalli, E. aeneus and *M. merluccius* RBCs consisted large intracellular bars that appeared to distort cell shape. It has been suggested that intracellular crystal formation is associated RBC deformations in all animal kingdoms including several groups of fishes [9]. Fish may respond physiologically to reduction in oxygen levels by changing the shape of erytrocytes as well as changing its haemoglobin properties [2,3]. The unusual appearance has been suggested to be a response to stressful handling and it is fully reversible [3]. It has been difficult to establish a meaningful explanation whether strenuous handling during capture or/and blood sampling affected for sickling process as two individuals of the same species differed even in tandem blood sampling, yet no sign of species-specificity. RBC sickling in toadfish was closely associated with the changes in intracellular pH as a consequence of capture stress or exhaustive exercise [3,10,11]. As the results of the present study were obtained from the natural populations no data existed on physiological conditions of the captured fish species. By a comparative study on the physiology and biochemical properties of different fish species erythrocytes, we better understand the occurrence and mechanisms of the sickling of fish RBC. When the mechanism how sickling in RBCs tolerated in several fish species is solved we hope that it may offer a valuable insight to human sickle-cell disease.

Acknowledgement

Special thanks to Assoc. Prof. Dr. Mehmet Cengiz DEVAL for trawl operation within his project (AUBAP2010.02.0121.026) in Antalya Bay. The present research note is a part of the study which has been still continuing. The study was partially presented as a poster in 1st International Symposium on Aquatic Sciences and Technology 15-17 May 2014, Girne - CYPRUS.

References

1. Glomski C A, Tamburlin J, Chaninai M. The phylogenetic odyssey of the erythrocyte. III. Fish, the lower invertebrate experience. Histol Histopath. 1992, 7(3): 501-528.

2. Herbing H. von and Cashon R. Hemoglobin sickling in Boreal fishes: Adaptation to the cold? Cardio-Respiratory Responses of Fish to Hypoxia, Hypercarbia and Temperature. 2004, 49-53.

3. Koldkjaer P, Berenbrink M. In vivo red blood cell sickling and mechanism of recovery in whiting, Merlangus merlangus. J Exp Biol. 210(19): 3451-3460.

4. Gregory TR. Animal Genome Size Database. 2014.

5. Abramoff MD, Magalhaes PJ, Ram SJ. Image Processing with ImageJ. Biophotonics International. 11(7): 36-42.

6. Schneider CA, Rasband WS, Eliceiri K W. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012, 9: 671-675.

7. Rasband WS. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. 2014.

8. Riccio A, Mangiapia G, Giordano D, Flagiello A, Tedesco R et al. Polimerization of hemoglobins in Arctic fish: *Lycodes reticulatus* and *Gadus morhua*. Life. 2011, 63(5): 346-354.

9. Harosi FI, Herbing H von, Keuran JR. Sickling of anoxic red blood cells in fish. Biol. Bull. 1988, 195(1): 5-11.

10. Nikinmaa M, Jensen FB. Blood oxygen transport and acid-base status stressed rainbow trout (Salmo gairdnerii): pre- and postbranchial values in winter fish. Comp. Biochem. Physiol. 1986, 84A: 391-396.

11. Knudsen P, Jensen FB. Effects of exhaustive exersize and

catecholamines on K+ balance, acid-base status and blood respiratory properties in carp. Comp Biochem. Physiol. 1998, 119(1): 301-307.