

Haematological and biochemical studies on selected species of fishes

Chuku LC and Uwakwe AA*

Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.

*uwadik@yahoo.com**

Abstract

In this study the following biochemical and haematological studies on Catfish (*Clarius gariepianus*); Mullet (*Mugi Cephalus*), Tilapia (*Oreochromis niloticus*), mudskipper (*Gobi I*), African sleeper (*Gobi-II*), were carried out; Haemoglobin electrophoresis, ABO Blood grouping and Glutathione-S-Transferase (GST) activity. About 50% of catfish indicated the Rh.D⁻ while the remaining 50% indicated the Rh.D⁺ blood group/. The 'A' 'O' and 'AB' blood groups were detected and glutathione-S-transferase activity values of 5.64 – 7.27 I.U were obtained. Hb concentration values were of the range of 5.5 -10.7gm% blood group results gave values of 50% D⁺ (A,O) and 50% (AB). GST activity values of 13.50-20.10 I.U were obtained. Gobi-I and Gobi-II Hb electrophoretic pattern was similar to human HbSS blood with Hb concentration range of 1.8-5.57gm%. GST activity values of 16.97-33.08 I.U. were obtained. Haemoglobin electrophoresis of Tilapia produced multiple patterns corresponding to human HbSS, HbCC and possibly HbCD. Hb concentration values were of the range of 3.70-12.94gm% and GST of 9.90-12.92 I.U were obtained. Only the 'O' and 'AB' blood groups were identified.

Key words: Haemoglobin, Glutathione-S-transferase, Genotype, Serum.

1. Introduction

Fishes are vertebrates and many species abound, majority the Hag-fishes (Myxini); Lampreys (Cephalaspidomorpha); Bony fish (Osteichthyes) and sharks and other cartilaginous fish (chondrichthyes) (Walter, 1997). As in other vertebrates, fishes possess vertebral column, or backbone and major sense organs. They also have the neural crest which gives fish the widely diverse features, including neurological cells; the cartilage of the gill bars' parts of the bony scales, including teeth (Walter, 1997). As in human blood, the cellular constituents of the fish blood are the red blood cells (erythrocytes), the white blood cells (leucocytes) and thrombocytes.

Some fish erythrocytes obtain their characteristic colour from haemoglobin, made-up of the colourless protein globin and the red-yellow pigment haem which contains iron; mature fish erythrocytes are relatively large and oval, with large nuclei. When viewed under a light microscope, the cytoplasm is light pink and the nucleus deep blue. The immature cells have blue cytoplasm and deep blue purple nucleus. These cells have more loosely packed chromatin. There is generally an inverse relationship between size and number of red blood cells (Christenson *et al.*, 1978).

Most studies of haemoglobin have been confined to the blood and muscle of mammals. As a result, the knowledge of the functional and structural properties of the haemoglobins of vertebrates is quite fragmentary. The respiration requirements of lower vertebrates vary over an enormous range. This gives rise to many interesting adaptations. At some extreme, the metabolic requirements for oxygen are so low that an oxygen transport pigment is unnecessary since sufficient oxygen dissolves physically into water.

The Antarctic ice fish have colourless blood and lack haemoglobin. At the other extreme, metabolically active fish often have such large requirements for oxygen that blood transport haemoglobin are not only necessary but even a slight anaemia might be a serious disadvantage. Like mammalian haemoglobin, fish exists in several molecular forms (Korpinicnikov, 1981) as multiple haemoglobin (polymorphic haemoglobin). Sick (1982) postulated the presence of two co-dominant allelic genes Hb11 and Hb12 which gives rise to the haemoglobin components Hb11 and Hb 12 respectively. The existence of several haemoglobin variants have been established in higher vertebrates (Korpinicnikov, 1981).

This work investigated, comparatively, some biochemical and haematological parameters on catfish, mullet, Tilapia and Mudskippers.

2. Materials and methods

Silver coloured giants species of fish of mean weight: 7.8 ± 1.05 kg were used, human antisera, distilled water, potassium cyanide, potassium ferricyanide, ethylene phosphate, di-sodium hydrogen phosphate, 1-chloro 2,4-dinitrobenzene, ethanol, reduced glutathione, cellulose acetate paper, Whiteman No. 3 filter paper, Tris-(hydroxymethyl) aminomethane, boric acid, applicator, sodium metabisulphite, phenylalanine, mountant, human blood (HbSS, HbAS, HbAA) and fish blood from different species of various lengths and weights (Table.1).

Table 1. Mean weights and lengths of fish species

Species	Mean weights (gms)	Lengths (cm)
Mullet	64.49 ± 0.56	18.00 ± 1.50
Tilapia	58.45 ± 1.25	14.00 ± 1.31
Catfish	84.64 ± 0.87	25.00 ± 1.25
Goby I (Africa sleeper)	44.00 ± 1.05	12.00 ± 0.85
Goby I (Mudskipper)	20.50 ± 1.32	11.00 ± 0.32

2.1 Collection of blood and preparation of lysates

The fishes used were properly washed to avoid contamination, using a syringe containing anticoagulant (EDTA), blood was collected from each fish by cardiac puncture and introduced into sample bottles duly labeled.

2.2 Blood grouping

The ABO and Rhesus blood grouping system are based on agglutination formation and procedure was as indicated in the kit for human blood grouping (Sigma product).

2.3 Haemoglobin grouping

This was estimated by the methaemoglobin method using Sahl's haemoglobinometer as described by Anosike et al. (1991).

2.4 Preparation of haemolysate

Red blood cells were separated from whole uncoagulated blood and suspended in volume of saline (0.9% NaCl) equivalent to the volume of displaced plasma. The cells were finally lysed by addition of two volumes of deionized water, exposing the haemoglobin.

2.5 Enzyme Assay of Glutathione-S-Transferase

The reaction of 1-chloro 3,4-dinitrobenzene (CDNB) with the thiol group of glutathione is catalyzed by Glutathione-S-transferase. The CDNB- glutathione conjugate absorbs light at 340nm and the activity of the enzyme is therefore estimated by measuring the changes in optical density (O.D.) at this wavelength as described by Anosike et al. (1991).

2.6 Determination of haemoglobin genotypes

Blood haemoglobin genotypes were determined using standard cellulose acetate electrophoresis as described by Anosike *et al.* (1991). Haemoglobin bands were identified by comparison with known human standards on same cellulose acetate strip. The rate of migration for the haemoglobins observed were in the order of HbA>HbF>HbS>HbC was identified as a double band or band running from HbA to HbS respectively.

2.7 Procedure for microscope slide sickling test

A drop of whole blood (e.g. HbSS) was placed on a slide and a drop of 2% solution metabisulphite was then added to it. This was properly mixed, sealed with the mountant, and incubated for 2hrs. The slide was observed with the microscope for possible sickling, fully sickled filamentous or spindled forms of HbSS predominated.

2.8 Procedure for polymerization inhibition test

This was done using the method described by Uwakwe and Akhidue (2000). Sodium metabisulphite (2%) and phenylalanine, followed by human or fish haemolysate were pipetted into a clean test tube separately. They were shaken and transferred into 1.0ml volume glass cuvette. The rate of polymerization was monitored spectrophotometrically at 700nm and optical density reading taken periodically. Changes in optical density per 2mins for 10mins at 700nm were recorded.

3. Results

3.1 Catfish

i) The haemoglobin concentration ranged from, 12.0-18.5g/ml. The detailed result is presented below (table 2). Furthermore, the same table shows other haematological and biochemical data arising from the experiment.

ii) ABO-RH: There is a random mixture of 'A' 'O' and 'AB' blood groups and Rhesus (\pm) factors similar to what is found in man. All catfish blood so far analysed behaved like the human HbAA blood. Fish haemoglobin identification was aided by the inclusion of human Hb standards.

iii) Glutathione-S-Transferase Activity: The Glutathione-S-transferase (GST) activity of catfish blood ranged from 5.64-7.27 I.U. The result is also presented on table. 2.

Table 2. The observed haematological parameters of genotyping, blood groups, Rhesus Factor, Hb and GST activity of catfish.

No.of fish	Genotype	Blood group	Rhesus factor	Hb (g/100ml) of blood	GST activity IU
Cat1	AA	A	D-	11.83	7.05
Cat2	AA	O	D-	16.26	5.77
Cat3	AA	O	D-	15.53	6.04
Cat4	AA	AB	D+	14.79	6.34
Cat5	AA	AB	D+	18.48	5.64
Cat6	AA	AB	D+	11.46	7.27
Cat7	AA	AB	D+	14.79	6.34
Cat8	AA	AB	D+	15.53	5.71
Cat9	AA	AB	D-	14.79	5.64
Cat10	AA	AB	D-	12.94	5.44
			X \pm SD	14.64 \pm 2.11	6.32 \pm 0.57

3.2 Mullet

i.) Using human antisera 'A' 'AB' and 'O', blood groups were identified. Both RhD+ (20%) and RhD- (80%) were present. The detailed result is presented below (table 3).

ii.) Haemoglobin concentration of mullet blood ranged from 6.0-11.0g/ml as indicated in table 3.

In all cases, Hb of mullet blood migrated in a manner comparable with that of human HbCC.

iii.) The GST activity of mullet blood ranged from 13.50 – 20.10 I.U.

iv.) Erythrocyte Sickling was difficult to achieve, similar to observation in human HbCC blood (see table.

3). Difficulty to sickle is a general attribute of the C-gene.

Phenylalanine increased survival time significantly ($p < 0.05$).

NOTE: 40 fishes were used per lot, ie, 10 fish for each of control, A,B and C, experiment done three times.

v). Survival time of mullet on exposure to sodium metabisulphite /phenylalanine solutions: Sodium metabisulphite (a strong deoxygenating compound) caused severe hypoxia, leading to faster death rate. However, the inclusion of phenylalanine, an antioxidant markedly improved the survival of fish (Table. 4).

3.3 Goby I and Goby II

i).Hb values (1.18-5.57g/ml) were much lower than those of the other fishes, Hb electrophoresis gave patterns resembling those of human HbSS.

ii).The 'A' 'AB' and 'O' blood groups were identified as shown in tables 5 and 6.

iii).The GST activities of Goby I and Goby II were fairly similar, ranging from 16.97-33.08 I.U. The results are also presented in tables 5 & 6.

Table 3. Sickled Erythrocytes of mullet on exposure to 2% sodium metabisulphite for 15 mins.

Fish No.	Sickled cell %
M1	1
M2	0
M3	1
M4	0
M5	2
M6	0
M7	0
M8	1
M9	2
M10	0

Table 4. Mullet survival time (Mins):In 2% sodium metabisulphite (A), 250µm phenylalanine (B) and 2% sodium-metabisulphite/phenylalanine mixture (C). Mean survival time in mins.

Fish lot	Control	A	B	C
1	100	48	120	60
2	116	53	130	80
3	200	70	300	95
Mean (mins)	141	57	182	78

Table 5. The observed haematological Parameters of Genotyping, Blood Groups, Rhesus Factor, Hb and G-S-T Activity of Mudskipper (Goby I).

No. of fish	Genotype	Blood group	Rhesus factor	Hb (g/100ml) Blood	G-S-T activity IU
Msk1	SS	AB	D-	5.57	18.90
Msk2	SS	AB	D+	2.36	22.08
Msk3	SS	A	D+	3.15	26.46
Msk4	SS	A	D+	3.07	16.97
Msk5	SS	AB	D+	1.18	16.49
Msk6	SS	AB	D+	5.57	18.91
Msk7	SS	A	D-	2.75	30.31
Msk8	SS	AB	D+	3.54	29.43
Msk9	SS	AB	D-	3.15	33.08
Msk10	SS	A	D+	2.36	26.49
			X ± SD	3.27 ± 1.38	23.91 ± 6.01

3.4 Tilapia

i) Hb concentration of Tilapia blood ranged from 3.79 - 12.94g/ml (Table 7). Hb electrophoresis indicated a wider variability in Hb migration. Both HbSS, HbCC and possibly HbCD were identified.

ii) Only the 'O' and 'AB' blood groups were identified. Both RhD⁺ and RhD⁻ were present (Table. 7).

iii) The GST activity of Tilapia blood samples ranged from 9.09-12.92 I.U (Table 7).

Table 6. The observed haematological parameters of genotyping, Blood Groups, Rhesus Factor, Hb and GST Activity of Goby-II (African Sleeper).

No. of fish	Genotype	Blood group	Rhesus Factor	Hb (g/100ml) Blood	GST activity IU
Goby1	SS	A	D+	2.36	18.90
Goby2	SS	AB	D+	3.15	22.08
Goby3	SS	AB	D-	3.07	26.46
Goby4	SS	O	D-	2.75	16.97
Goby5	SS	O	D+	2.36	16.49
Goby6	SS	AB	D+	3.15	18.91
Goby7	SS	AB	D-	1.97	30.31
Goby8	SS	B	D+	3.54	29.43
Goby9	SS	A	D+	3.74	33.08
Goby10	SS	B	D+	2.75	26.49
			X ± SD	2.88±0.55	22.93 ± 2.28

Table 7. Observed results of genotyping, blood groups, Rhesus factor, Hb and GST activity of Tilapia.

No. of fish	Genotype	Blood group	Rhesus factor	Hb (g/100ml) Blood	GST activity IU
T1	SC	O	D-	10.35	12.00
T2	SC	O	D+	11.46	10.09
R3	SC	O	D+	12.94	11.27
T4	SS	AB	D-	3.70	11.26
T5	SC	O	D+	11.09	12.27
T6	SS	O	D+	12.20	12.80
T7	SD	AB	D-	8.87	12.82
T8	SC	AB	D+	10.72	11.66
T9	SS	O	D-	9.24	12.40
T10	CC	AB	D+	12.20	11.96
			X ± SD	10.28± 2.65	11.83±0.84

4. Discussion

Hematological and biochemical studies of fishes will afford an opportunity for a better understanding of the phylogenetic relationship in vertebrate animals.

A common function of blood is oxygen mobilization which is based on the common structure of the haemoglobin molecule (Walter, 1997). Therefore, it is an interesting challenge to tailor the blood of fishes or other lower vertebrates into prospective researches in a bid to discover the presence of certain genes with phenotypical expressions of interest that will intimately serve man. Also a major advantage here is that by using fishes or other lower vertebrates as research models moral and human ethical problems are resolved. The study of sickle cell haemoglobinopathy is a case in point as some other vertebrates exhibit similar characteristics to human haemoglobin polymorphs. Table 7. for instance shows that the work done on haemoglobin electrophoresis experiments on catfish demonstrated the presence of human haemoglobin A-type haemoglobins. Human haemoglobin SS-type was identified in mudskipper (Goby-I) and African Sleeper (Goby-II)

It would however, be very naive at this stage to assume conclusively about the exact similarity of these non-human hemoglobin variant to those of human as definite conclusions are only possible when other biochemical techniques such as globin sequencing is performed. But migration patterns of the hemoglobin of these fishes are very revealing and obviously point to similarities in the

charge properties of the hemoglobin polypeptides of man.

Therefore, as a further confirmation of the possible nature of these human haemoglobin types, the HbSS, HbCC, and HbSC-type erythrocytes of mudskipper (Goby I), African Sleeper (Goby II), mullet and Tilapia were subjected to sickling using 2% sodium metabisulphite to induce hypoxia. The results proved quite a spectacle in that results were similar to human blood. The HbSS-type erythrocytes of Goby-I and Goby-II sickled phenomenally as human HbSS erythrocytes. Indeed sickling was difficult to achieve using the HbCC and HbSC-type erythrocytes of mullet and tilapia similar to observations in human.

But when mullet (whole fish) was exposed to 2% sodium metabisulphite solutions, the strong deoxygenating compound caused hypoxia, leading to faster death rate. There was however, an improved survival following the inclusion of phenylalanine, an antioxidant in the solution. These results point to a possible increased oxidant stress in HbCC erythrocytes which is further aggravated by the exposure to the deoxygenating compound, sodium metabisulphite. The possibly raised oxidant stress in the HbCC-type erythrocytes was further confirmed by the high GST activity (13.50-20.10) observed in blood of mullet. High GST activity has been shown to be a possible marker of increased erythrocyte oxidant stress (Anosike *et al.*, 1991).

Goby-I and II with HbSS-type erythrocytes were also noted to have elevated GST activity (16.97-33.08 I.U) compared to a value of (5.64 - 7.37 I.U) obtained for catfish with HbAA-type erythrocytes (table. 2) in a bid to further establish similarities in the blood tissues of vertebrates. The blood groups of the various vertebrate animals were determined using human antisera standards.

Surprisingly, the presence of blood groups similar electrophoretically to human blood groups 'A', 'O', 'AB' and 'B' as well as Rhesus factors D⁺ and D⁻ were obtained in catfish, mullet, Goby I and II, tilapia gave blood 'AB' and 'O' and Rhesus factors D⁺ and D⁻. These results point to obvious similarities in the erythrocyte antigenic determinants of the lower vertebrate animals and those of humans.

5. Conclusion

In conclusion, this work has demonstrated the following:

- a) The presence of human haemoglobin-type polymorphic genes in fishes.
- b) Possible similarities in blood groupings of human and fishes.
- c) A relationship between GST activity and haemoglobin genotype in fishes as the case in humans. (Anosike *et al.*, 1991).
- d) A relationship between haemoglobin concentration and haemoglobin- type in the fishes studies in which the presence of haemoglobinopathic type gene were determined.
- e) It is hoped that these findings would prove useful towards the provision of experimental models for the study of human genetic disorders. It is also possible that these findings could prove a springboard for the better understanding of the vertebrate evolutionary tree.

6. References

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