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Haematological and Biochemical Studies on some Species of Fishes

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ABSTRACT: In this study the following biochemical and haematological studies on Catfish (clarius gariepianus); Mullet (Mugi Cephalus), Tilpia (Oreochromis niloticus), mudskipper (Gobi I), African sleeper (Gobi-II), were carried out; Haemmoglobin electrophoresos, ABO Blood grouping and Glutathine-S-Transferase (GST) activity. Catfish haemoglobin electrophoretic pattern was similar to human HbAA blood, with haemoglobin (Hb) concentration range of 12.0 – 17.8gm%. About 50% of catfish indicated the Rh.D$^-$ while thew 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. Haemoglobin electrophoresis of mullet gave a pattern similar to HbCC of human blood. 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 opf 9.90-12.92 I.U were obtained. Only the 'O' and 'AB' blood groups were identified. @JASEM

KEY WORDS: Haemoglobin, Glutathione-S-transferase, Genotype, Serum.

Fishes are vertebrates and many species abound, majority the Hag-fishes (Myxini); Lampreys (Cephalaspidmorphi); Bony fish (Osteichththeyes) and sharks and other cartilaginous fish (chondrichthyes) (Walter, 1997). As in other vertebrates, fishes posses 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 microsope, 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 are 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. Fish haemoglobin, like mammalian, exits 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 haemotological parameters on catfish, mullet, Tilapia and Mudskippers.

MATERIALS AND METHODS

Silver coloured giants species of fish of mean weight: 7.8 ± 1.05kg 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 (table1).

Collection of Blood and Preparation of Loysates: 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 duely labeled.

Blood Grouping: The ABO and Rhesus blood grouping system are based on agglutination formation

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and procedure was as indicated in the kit for human blood grouping (Sigma product).

Haemoglobin Grouping: This was estimated by the methaemoglobin method using Sahl's haemoglonometer as described by Anosike *et al.*(1991).

*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.

Table 1: mean weights and lengths of fish species used

SPECIES	MEAN WEIGHTS IN GRAMS	LENGTHS (mm)
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
AFRICA SLEEPER	44.00 ± 1.05	12.00 ± 0.85
MUDSKIPPER	20.50 ± 1.32	11.00 ± 0.32

Enzyme Assay of Gluthathione-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).

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.

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 moutant, and incubated for 2hrs. The slide was observed with the microscope for possible sickling, fully sickled filamentous or spindled forms of HbSS predomdinated.

*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.

RESULTS AND DISCUSSION

Results for 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 haemotological and biochemical data arising from the experiment.

ii). ABO-RH: There is a random mixture of 'A' 'O' and 'AB' blood groups and Rhesus (±) 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) Gluthathione-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 1.

Results for 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 (see table 3).

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.

*Results for 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. The data are as presented in table 5.

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 and 6.

Table 1: The Observed Haemotological Parameters of Genotyping, Blood Groups, Rhesus Factor, Hb and G-S-T Activity of Catfish

No. of fish	Genotype	Blood Group	Rhesus Factor	Hb (g/100ml) of blood	G-S-T activity i/u
Cat_1	AA	A	D-	11.83	7.05
Cat_2	AA	O	D-	16.26	5.77
Cat_3	AA	O	D-	15.53	6.04
Cat_4	AA	AB	D+	14.79	6.34
Cat_5	AA	AB	D+	18,48	5.64
Cat_6	AA	AB	D+	11.46	7.27
Cat_7	AA	AB	D+	14.79	6.34
Cat_8	AA	AB	D+	15.53	5.71
Cat_9	AA	AB	D-	14.79	5.64
Cat_{10}	AA	AB	D-	12.94	5.44
			X ± SD	14.64 ± 2.11	6.32 ±0.57

Table 3: Shows (%) sickled Erythrocytes of mullet on exposure to 2% sodium metabisulphite for 15 mins

FISH NO.	% SICKLED CELLS
M_1	1
M_2	0
M_3	1
M_4	0
M_5	2
M_6	0
M_7	0
M_8	1
M_9	2
M_{10}	0

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

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

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).

Table 5: The observed haematological Parameters of Genotying, 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 i/u
Msk_1	SS	AB	D-	5.57	18.90
Msk_2	SS	AB	D+	2.36	22.08
Msk_3	*SS*	A	D+	3.15	26.46
Msk_4	*SS*	A	D+	3.07	16.97
Msk_5	*SS*	AB	D+	1.18	16.49
Msk_6	*SS*	AB	D+	5.57	18.91
Msk_7	*SS*	A	D-	2.75	30.31
Msk_8	*SS*	AB	D+	3.54	29.43
Msk_9	*SS*	AB	D-	3.15	33.08
Msk_{10}	*SS*	A	D+	2.36	26.49
			X ± SD	3.27 ± 1.38	23.91 ± 6.01

Table 6: The Observed Haematological Parameters of Genotyping, Blood Groups, Rhesus Factor, Hb and G-S-T Activity of Goby–II (African Sleeper)

No. of fish	Genotype	Blood Group	Rhesus Factor	Hb (g/100ml) Blood	G-S-T activity i/u
$Goby_1$	SS	A	D+	2.36	18.90
$Goby_2$	SS	AB	D+	3.15	22.08
$Goby_3$	SS	AB	D-	3.07	26.46
$Goby_4$	SS	O	D-	2.75	16.97
$Goby_5$	SS	O	D+	2.36	16.49
$Goby_6$	SS	AB	D+	3.15	18.91
$Goby_7$	SS	AB	D-	1.97	30.31
$Goby_8$	SS	B	D+	3.54	29.43
$Goby_9$	SS	A	D+	3.74	33.08
$Goby_{10}$	SS	B	D+	2.75	26.49
			X ± SD	2.88±0.55	22.93 ± 2.28

*Results for 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 1.7). iii).The GST activity of Tilapia blood samples ranged from 9.09-12.92 I.U (Table 7).

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	G-S-T activity i/u
T_1	SC	O	D-	10.35	12.00
T_2	SC	O	D+	11.46	10.09
R_3	SC	O	D+	12.94	11.27
T_4	SS	AB	D-	3.70	11.26
T_5	SC	O	D+	11.09	12.27
T_6	SS	O	D+	12.20	12.80
T_7	SD	AB	D-	8.87	12.82
T_8	SC	AB	D+	10.72	11.66
T_9	SS	O	D-	9.24	12.40
T_{10}	CC	AB	D+	12.20	11.96
			X ± SD	10.28± 2.65	11.83±0.84

Haematological 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 7.1 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) (see tables 5 & 6).

It would however, be very naive at this stage to assume conclusively about the exact similarity of these non-human haemoglobin 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 haemoglobins of these fishes are very revealing and obviously point to similarities in the charge properties of the haemoglobin polypeptides of man.

Therefore, as a futher 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 phenylalalnine, 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 (tables 1, 5 and 6) 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.

*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.

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