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Observation on Haematological and Enzymatic Changes on Spontaneous Papillomatous Condition of A*nabas testudineus* (Bloch)

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Abstract

Haematological and enzymatic studies were made on the spontaneous cases of skin papilloma of climbing perch (*Anabas testudineus*). It was revealed that blood parameters and enzymatic levels showed hyperactive immune system in papilloma-affected fish as compared to the control group. Marked elevation of serum enzymatic pattern i.e. alkaline phosphatase (ALP), acid phosphatase (ACP), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in spontaneous cases of tumour as compared to control.

Introduction

Tumours of the skin in fish are most frequently reported. Benign tumours of epithelial origin occur all over the world on many species of fish from both freshwater and marine habitats. Papillomas in fish vary in size and shape from a low elevation to extensive nodular leaf like tissue projecting above the skin surface. Papilloma in climbing perch (*Anabas testudineus,* Anabantidae) (Local name: koi) has been reported (Fiebiger 1909, Nigrelli 1952, Pal and Tripathi 1978, Sarkar and Dutta Choudhury 1953). This work was carried out to record the changes associated with the blood parameters and enzyme activities in spontaneous cases of climbing perch.

Materials and Methods

Eleven climbing perch (average weight 25 g) having tumours on their bodies were collected from the swampy area of Puri district, Orissa, India and brought to the laboratory and reared in fiberglass tanks of 40 l capacity under aeration and fed with normal diet. The clinical signs were recorded for three weeks. The size and weight of the tumours were also recorded. After 10 days, blood/serum samples were collected for analysis of RBC count, WBC count, blood haemoglobin and serum protein, alkaline phosphatase (ALP), acid phosphatase (ACP), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) enzymatic activity and biochemical parameters like serum triglyceride, cholesterol and glucose. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined using Neuber's haemocytometer with Toission's solution as diluting fluid for TEC and Tork's solution for TLC. Haemoglobin percentage was determined by using Shali's haemometer. Blood glucose level was estimated following the method of Schmidt (1971). Two ml of reagent solution containing buffer, enzymes 4-amoinophenazone, phenol and standard glucose (1 $mgml^{-1}$) were taken in a clean and dry test tube and 20 µl of the blood was added. Simultaneously a standard and a blank reading was taken (2 ml each). It was mixed thoroughly and incubated at 25°C for 30 min. After the incubation period absorbance was taken at a wave length of 546 nm using Boehringer Mannheim photometer-4010 model. The glucose activity was expressed as $mgdl^{-1}$. The final concentration was calculated as: $C=100 \times A_{sample} / A_{standard,}$ Where A_{sample} = Absorbance of sample, $A_{standard}$ = Absorbance of standard. Serum protein was estimated using Boehringer-Manheim kit. Protein was precipitated from the sample by trichloroacetic acid and then determined by Biuret method (Henry 1964). Blood smears were prepared over a grease free clean slide and air-dried. Subsequently, the slides were stained with Wright's Giemsa (Hasser 1960). The alkaline phosphatase and acid phosphatase activity were determined following the procedure of Jafee and Badansky (1943). Two ml of reagent solution containing citrate buffer (150 mmol), napthylphosphate (12.1 mmol), fast red TR-salt (1.2 mmol), pentane-di-ol (220 mmol), tartarate (100 mmol) and acetic acid (0.8 mmol) was taken in a clean and sterile test tube. Then 200 µl serum was added to it. The mixture was shaken thoroughly and incubated at 37°C for 5 minutes and initial absorbance was recorded at 405 nm in a photometer (Boehringer Mannheim photometer 4010 model) and subsequently three recordings were taken at exactly one-minute interval using a stopwatch. The ACP activity was expressed as Ul^{-1}. The final concentration was calculated as: $Ul^{-1} = 122\ \Delta A_{sample}$ at 405nm/min.,

where ΔA is change in absorbance per minute. For estimation of ALP, reagent solution (3ml) containing diethanolamine buffer, $MgCl_2$ and *P*-nitrophenyl phosphate was taken in a clean and sterile test tube. To it 50 µl serum was added and mixed thoroughly. The mixture was incubated at 37°C for one minute and absorbance was recorded as that ALP. The final concentration was calculated in Ul^{-1} = 3300 X ΔA_{sample} at 405 nm/min. GPT activity was determined using Boehringer Manheim kit as per the methods of Bergmeyer and Horder (1980). Two ml of reagent solution containing Tris-buffer, L-alanine, LDH enzyme and NADH enzyme were taken in a clean and sterile test tube and 200 µl of the serum was added. The whole mixture was incubated at 25°C for 1 min and then 200 µl of α-oxyglutarate was added to it. The solution was mixed thoroughly and initial absorbance was recorded at 340 nm wavelength using a Boehringer Mannheim photometer 4010 model and three subsequent readings at 1 min intervals. The GPT activity was expressed Ul^{-l}. The final concentration was calculated as: Ul^{-l} =1905 × A 340 $nmmin^{-1}$, where 1U = 16.67 × 10^{-3} µ Kat. GOT activity was analysed as per the methods of Wallnofer et al (1978). Initially 2 ml of reagent solution containing Tris buffer, L-Asparate MDH, LDH, NADH enzymes was taken in a clean and sterile test tube, then 200 µl of serum was added to it. It was mixed thoroughly and incubated for 1 min at 37°C after which 2 ml of L-Oxyglutarate was added. The solution was mixed thoroughly and initial absorbance was recorded and subsequently three readings were taken at 340 nm wavelength using Boehringer Mannheim photometer 4010 model at exactly 1 min interval using a stop watch. The GOT activity is expressed Ul^{-l}. The final concentration was calculated as: Ul^{-l} =1905 × A 340 $nmmin^{-1}$, where 1U = 16.67 × 10^{-3} µKat and A= Absorbance. Serum cholesterol and triglyceride were determined as per the methods of Trinider (1969). For estimation of cholesterol activity, reagent solution (2ml) containing Tris buffer, Magnesium aspirate, 4-aminophenazole, Sodium chloride, Phenol, 3-4 dichlorophenol, hydroxy polyethoxy-n-alkanes, cholesterol esterase, cholesterol oxidase, and peroxidase were taken in a test tube. Then 200 µl serum was added and mixed thoroughly. The mixture was incubated for 5 minutes at 37°C and absorbance was recorded at 546 nm wavelength. The cholesterol activity was expressed as $mgdl^{-1}$ and calculated C ($mgdl^{-1}$) = 853 X $A_{sample}/A_{standard}$, where A_{sample}=Absorbance of sample and $A_{standard}$=Absorbance of standard. For estimation of triglyceride, reagent solution (2 ml) containing ATP, 4-aminophenazone lipase, glycerol phosphate oxidase, glycerolinase, peroxidase and 4-chlorophenol was taken in a test tube and 20 µl serum was added to it. The solution was mixed thoroughly and incubated for 10 minutes at 25°C and concentration was measured at a wavelength of 546 nm. Triglyceride activity

was expressed as $mgdl^{-1}$. The total concentration was calculated as C ($mgdl^{-1}$) = 1040 x A_{sample}. Simultaneously control samples from healthy climbing perch were analyzed for the above-mentioned parameters.

Results

Affected fishes showed listlessness and aberrant swimming behaviour. Grossly tumours were observed all over the body. However, the majority had tumours mostly on their head region. The size of the tumours varied from 1 to 10 mm with a depth of 1mm to 5 mm. The tumours were grey to steel grey in colour. On autopsy there was no ascitic fluid. The liver was pale while kidney was slightly hypertrophied. Kidney squash showed high neutrophil content. The biochemical parameters of the blood/serum and enzyme activity of the serum of the control and affected are presented (Table 1).

Behaviour of an organism represents the final integrated result of a diversity of biochemical and physiological process (Warner et al 1966). Disorders in the internal anatomy are reflected by clinical manifestation. Aberrant swimming and loss of equilibrium make the fish conspicuous in the surroundings. More slime on the skin surface indicates that the fish was more susceptible to disease process. Blood parameters and enzyme levels showed hyperactive immune system in papilloma affected fish as compared to the control group (Table 1). Marked differences in the values of blood parameters especially with regard to the white blood cells were observed. The red cell to white cell ratio was reduced in the diseased ones, which reflects a drop in red cell count than a rise in the number of white cells. This drop in turn reflected in the lower haemoglobin concen-

Table 1. Haematological and enzymatic parameters of climbing perch (*Anabas testudineus*) with epidermal papilloma

Parameters	Control fish	Infected fish
RBC cells/mm^3	$1.06x10^6$-$1.95x10^6$	$0.58x10^6$-$0.79x10^6$
WBC cells/mm^3	$6.8x10^4$-12.2 $x10^4$	7.9 x 10^4-15.4x50^4
Red/White cell ratio	15.58-15.98	5.09-7.34
Hb (gm%)	7.2-11.5	.6-7.2
Glucose ($mgdl^{-1}$)	448-602	199.0-240.0
Total Serum Protein (gdl^{-1})	7.91-8.72	2.52-4.28
Triglyceride ($mgdl^{-1}$)	101-127	40.56-48.32
Cholesterol ($mgdl^{-1}$)	135.0-138.42	139.89-145.42
Alkaline phosphatase (Ul^{-1})	0.643-0.923	1.929-2.345
Acid phosphatase (Ul^{-1})	3.33-6.82	13.2-16.37
Glutamate phosphate Transaminase (Ul^{-1})	1.095-3.25	3.285-5.82
Glutamate oxidase transaminase (Ul^{-1})	2.19-4.62	3.28-6.34

tration. Not only the number of red cells but also their nature is changed in the affected climbing perch. In fixed smears it was noticed that many circulating red cells were immature, as evidenced by their more round shape and their staining properties (Fig. 1). The authors could not come across any previous references on papilloma induced haematological changes excepting the work of Mulcahy (1975) who had reported a nearly similar observation while working with lymphoma.

Reduction in the total serum protein levels was observed in pike with lymphomas (Mulcahy 1975). According to him this might be attributed due to renal excretion of blood protein thereby reducing in the blood. In our present observation, the serum protein was drastically reduced. It might be presumed that in case of prolonged and continued papillomatous condition, the deleterious effect of the tumour on the protein synthesis and kidney function might be profound which accounts for the progressive reduction in the concentration of the total serum protein. Blood glucose and triglyceride values were also reduced drastically. The cholesterol value was not altered as compared to the control. There was a marked elevation of serum enzyme pattern i.e. ACP, ALP, GOT and GPT in spontaneous cases as compared to control (Table 1). An increase in the enzyme activity in blood serum was observed in mammals in pathologic states, pointing to disturbances in liver function (Hanke and Piotrowski 1980). However, reports on increase in ACP, ALP, GOT and GPT due to

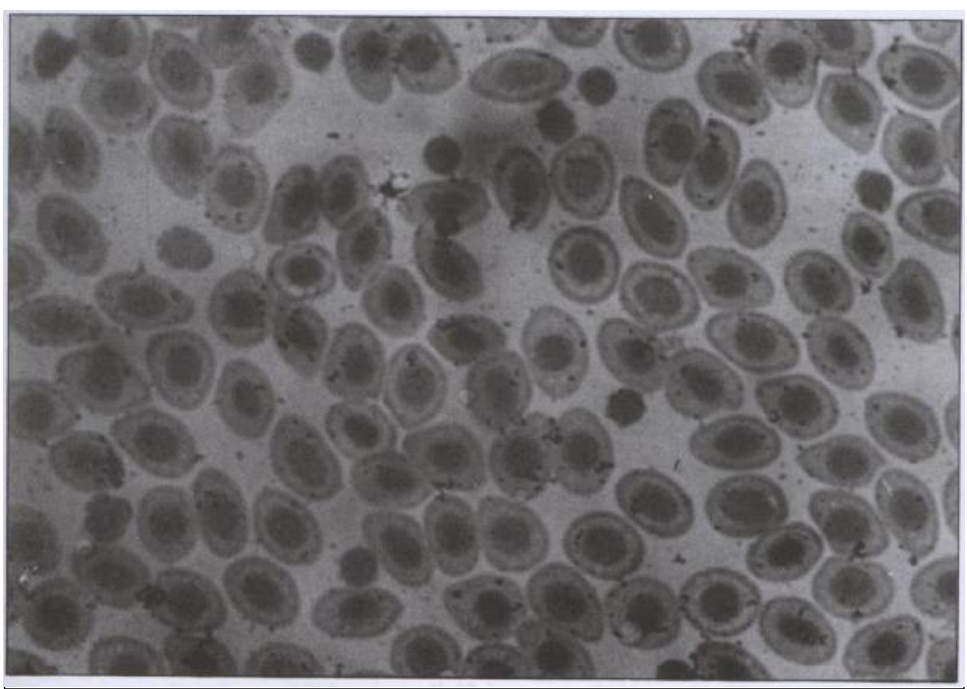

Fig. 1. Blood smear of *A. testudineus* showing immature red blood cells

papilloma tumours are not available. It may be concluded that skin papilloma could alter the activities of some of the blood parameters as well as enzyme activities as evidenced in the present study. In chronic cases the fish becomes sufficiently weak due to the altered profile of the blood and enzymes and make the fish stressed.

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