Assessment of Micronucleation and Nuclear Abnormalities in Deformed *Heteropneustes fossilis* Fish: Implications for Genotoxicity and Environmental Health

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Abstract: ***The objective of the present study was to detect the frequency of micronucleation and nuclear abnormalities in peripheral erythrocytes of deformed fish (Heteropneustes fossilis Bloch) collected from the market of West Bengal. As per visual observation of abnormal shape of fish specimens, the samples were collected. The morphological parameters viz. head length (HL), head width (HW), body length (BL), body width central position (BW), caudal fin length (CFL) and caudal fin width (CFW) were measured manually. The micronucleation (MN) and nuclear abnormalities (NAs) tests was performed in the peripheral erythrocytes under bright field microscope (400X magnification). The present result indicates an alarming risk of genotoxicity due to the formation of MN and NAs such as blebbed nuclei (BLN), notched nuclei (NN), vacuolated cytoplasm (VC), fragmented nuclei (FN), nuclear cariolysis (NC), dumble shaped nuclei (DSN), retracted nuclei (RN) and binuclei (BN) in the peripheral erythrocytes of deformed fish specimens. It is suggested in future investigation to know the cause of deformities and nuclear abnormalities as per the accumulation study of heavy metals and/or organic compounds.***

Keywords: Deformed fish, Environmental factors, Freshwater fish, Market fish, *Heteropneustes fossilis*, Abnormal nucleation

1. Introduction

In tropical Asia, the freshwater ecosystems harbour the rich faunal species diversity especially fish species. The diverse groups of fishes are also developed in the wide range of morphological, behavioural, and life history attributes, which is characterized the constituent species in which the different habitats are implanted in inland waterbodies. [1]

In the case of morphological anomalies were observed in fish species such as *Oreochromis* sp., *Mozambique tilapia*, *Catla catla*, *Barbus barbus*, *Bariliusbendelisis*, *Cirrhinusmrigala*, *Puntius sarana*, *Tor putitora*, *Heteropneustes fossilis*, *Mystusbleekeri*, etc. Several studies have emphasized different types of morphological abnormalities viz. fin erosion, fin deformity, lower lip protrusion, gill deformity, ocular disorders, scale deformity and disorientation, neoplasia or hyperplasia, etc. in fish collected from contaminated waters. [2 - 10]

Hussain et al. [11] also reported single and double micronucleation in the peripheral erythrocytes of fish, *Labeorohita*. Mandal [12] reported about induction of micronuclei (MN) in the fish (*Mystuscavasius*) erythrocytes of inhabited in the river Hooghly near Birlapur, Batanagar and Budge Budge, West Bengal. Moreover, the higher genotoxicity resulted loss of population of fish specimens. Hussain et al. [11] conducted a comparative MN bioassay between two fish species *Wallago attu* and *Cirrhinusmirigala* to know the freshwater pollution. Mondal et al. [13] evaluated nuclear abnormalities (NAs) in the peripheral erythrocytes of fish (*Liza parsia*) inhabited in Sundarban coastal zone, West Bengal and reported alarming genotoxic risk. In a recent study, Gupta & Talapatra [14] tested MN and NAs in the peripheral erythrocytes of two fish species (*Labeo* bata and *Orechromis* sp.) inhabiting East Kolkata Wetlands (EKWs) but did not observe alarming risk of genotoxicity, which may be due to COVID - 19 lockdown.

According to the investigators, many causative factors are well known such as deficiency of dietary levels of vitamin A, the presence of oily surface film, high swimming effort, temperature variation, water current, unfavourable highly unsaturated fatty acids dietary levels, metals and metalloids, etc. in different fish species. [15 - 23]

It was evaluated to detect MN and NAs in peripheral erythrocytes of deformed fish (*Heteropneustes fossilis* Bl.) collected from local market.

2. Materials and Methods

Fish species

Five fish specimens, *Heteropneustes fossilis* were collected from the local fish seller of wholesale market of Haridevpur, West Bengal. Just died fish of 5 species were selected for the study.

External morphological features

All five fish specimens were observed visually for abnormal external gross morphology. The gross abnormal morphology was studied as body length (BL in cm), body weight (BW in gm), Head length (HL in cm), Head width (HW in cm), body width central position (BWcp in cm), caudal fin length (CFL in cm) and caudal fin width (CFW in cm). Beside these, any curvature on neck, trunk, and tail region and any deformity in caudal fins through visual observation as per protocol of Sun and Tsai [3]and Fragkoulis et al. [8]

Genotoxicity study:

The blood was collected from heart by using insulin syringe. Total five fish samples (*H. fossilis*) were studied for genotoxicity with special reference to MN and NA. After collection of blood the smear was prepared onto slide per fish. All the slides were dried at room temperature and kept in slide box for MN and NA assay. MN and NA frequencies in the peripheral erythrocytes of was evaluated according to the method of Fenech. [24] All the smeared slides were fixed in 100% methanol for 10 min. followed by staining with Leishman solution for 10 min, airdried and then prepared for permanent use. Total 1000 erythrocytes per slide were examined and 1000 nos. of erythrocytes were scored for each specimen under a brightfield microscope with oil immersion at 400X magnification. MN was identified as per criteria described by Fenech et al. (2003). Other nuclear anomalies (NA) such as blebbed nuclei (BLN), notched nuclei (NN), vacuolated cytoplasm (VC), fragmented nuclei (FN), nuclear cariolysis (NC), dumble shaped nuclei (DSN), retracted nuclei (RN) and binuclei (BN) were scored separately, as per the criteria described by Da Silva Souza and Fontanetti [25] followed by the protocol of Mandal, [12] Mondal et al. [13]and Gupta and Talapatra. [14]

3. Results

Table 1 evaluates the morphometric analysis of *H. fossillis*, whichrevealed that BL, BW, HL, HW, BWcp, CFL and CFW ranged between 18 - 32 cm, 42 - 160 gm, 2.2 - 4.0 cm, 2.0 - 4.9, 5.3 - 8.0 cm, 2.2 - 4.4 and 2.0 - 3.5 cm, respectively. The abnormal morphological features viz. swollen near cervical region, followed by compression before caudal fin and the fin is asymmetrical (not homocerecal), rapture fin rays were observed (Fig 1).

Table 1: Morphological deformities of fish *H. fossilis*

Deformed fish specimens	BL (in cm)	BW (in gms)	HL (in cm)	HW (in cm)	BWcp (in cm)	CFL (in cm)	CFW (in cm)
Specimen 1	30.0	150.0	2.2	2.1	8.0	2.2	2.0
Specimen 2	31.0	160.0	4.0	4.9	5.3	3.0	2.0
Specimen 3	19.0	45.0	2.5	2.2	8.0	4.4	3.5
Specimen 4	18.0	42.0	2.7	2.4	5.3	3.8	2.5
Specimen 5	32.0	155.0	2.5	2.0	8.0	2.7	1.7

cm = Centimeter; gms = Grams; BL = Body length; BW = Body weight; HL = Head length; HW = Head width; BWcp = body width central position; CFL = Caudal fin length; CFW = Caudal fin width

Figure 1: Photographic representation of morphological deformities of fish *H. fossilis*

Table 2 evaluates the frequencies (%) MN and NA values (Mean ± SD) in the peripheral erythrocytes of *H. fossilis*. In the case of MN frequencies (%), the value was observed 1.30 ± 0.12. For frequencies (%) of NA such as BLN, NN, VCFN, NC, DSN, RN, and BN values were obtained in the fishes as 2.78 ± 0.22, 0.28± 0.06, 2.80± 0.24, 1.26± 0.08, 0.42 ± 0.15, 1.20± 0.19, 1.38± 0.10 and 0.41 ± 0.20, respectively. The highest frequencies (%) of NA were obtained in the case of VC followed by BLN, RN, FN and DSNwhile minimum frequencies (%) were obtained for NC, BN and RN. Fig 2 represents the photomicrographs of MN and NAs in the peripheral erythrocytes of *H. fossilis*.

Table 2: Percentage frequencies of MN and NAs in the peripheral erythrocytes of fish *H. fossilis* (Mean ± SD; n = 5)

MN	NAs							
	BLN	NN	VC	FN	NC	DSN	RN	BN
1.30 ± 0.12	2.78 ± 0.22	0.28 ± 0.06	2.80 ± 0.24	1.26 ± 0.08	0.42 ± 0.15	1.20 ± 0.19	1.38 ± 0.10	0.41 ± 0.20

MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blebbed nuclei; NN = Notch nuclei; VC = Vacuolated cytoplasm; FN = Fragmented nuclei; NC = Nuclear cariolysis; DSN = Dumble shaped nuclei; RN = Retracted nuclei; BN = Binuclei

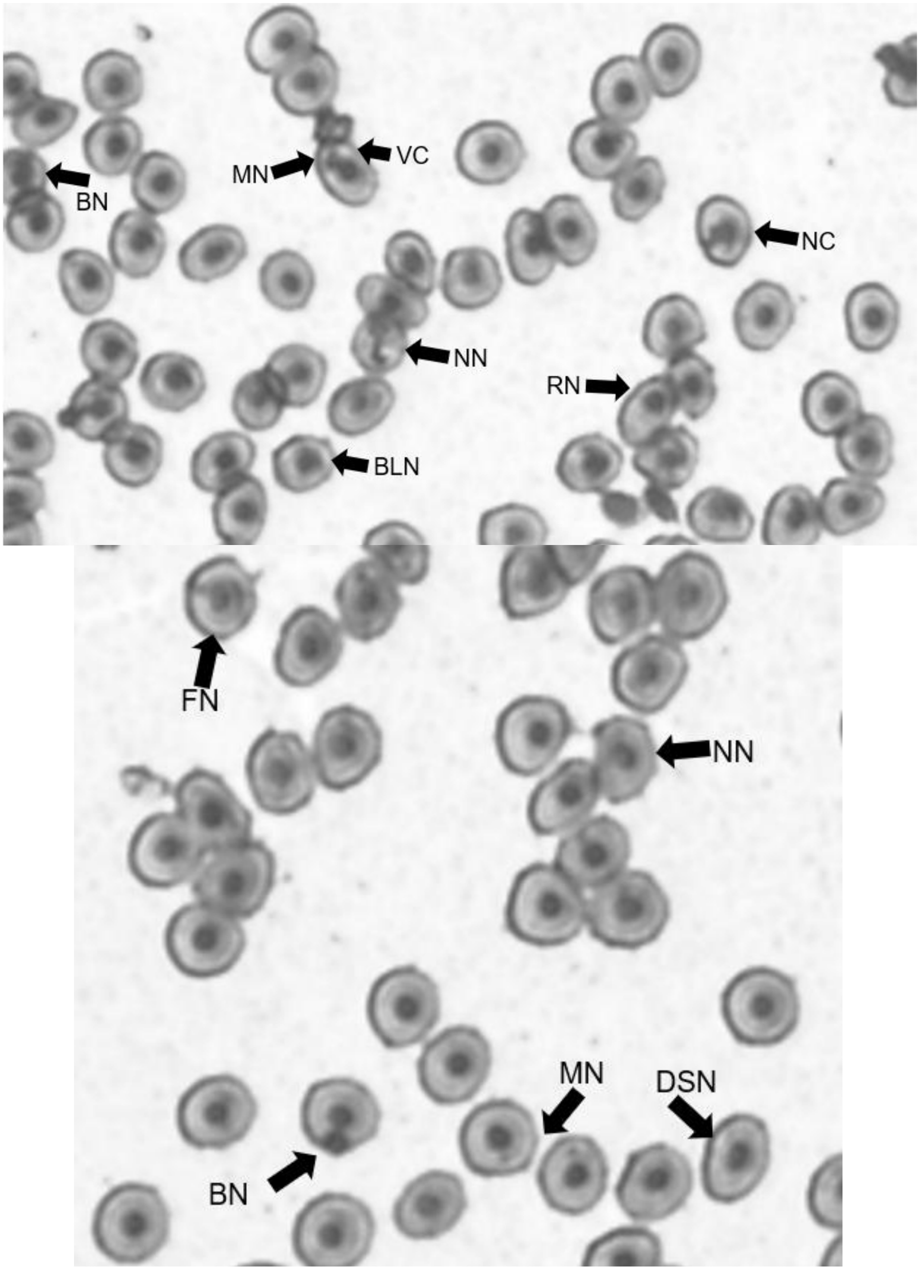

Figure 2: Microphotographs (400x magnification) of MN and NAs in the peripheral erythrocytes of *H. fossilis* (MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blebbed nuclei, NN = Notch nuclei; VC = Vacuolated cytoplasm; FN = Fragmented nuclei; NC = Nuclear cariolysis; DSN = Dumble shaped nuclei; RN = Retracted nuclei; BN = Binuclei)

4. Discussion

The present study evaluated abnormal morphology and genotoxicity with special reference to MN and NA assay in the peripheral erythrocytes of five freshwater fish specimen (*H. fossilis* Bl.) collected from fish market.

Several studies indicated that these abnormalities cause due to several factors viz. water pollution, change inphysico - chemical properties of the habitat, malnutrition, injuries from trauma, genetic factor, etc. [8, 16, 26 - 31]

In the present study, morphological abnormalities were observed mainly body shape affected such as swollen near cervical region, followed by compression before caudal fin along with asymmetrical shape (not homocerecal), rapture fin rays in *H. fossilis*. Some similarities were observed in the previous studies with fish species viz. *Danio rerio, Clarias gariepinus, Ameiurus nebulosus, Labeorohita, Cirrihinusmrigala, Catla catla*. [5, 7, 9, 32, 33] Herein, we did not study any causative factors.

It is well established fact that many environmental factors viz. temperature, metals, and metalloids, etc. pose genotoxicity in the peripheral erythrocytes of fish species. [12

- 14, 34 - 36] In the present study, the causative factor of genotoxicity is unclear but morphologically deformed fish pose genotoxicity after induction of MN and NAs.

5. Conclusion

Through an observational study on five fishes (*H. fossilis* Bl.), it was recorded that abnormal morphology like swollen near cervical region, followed by compression before caudal fin and the fin is asymmetrical (not homocerecal), rapture fin rays may be suitable indicator of the alteration of habitat. Moreover, this abnormality was also recorded in the peripheral erythrocytes of studied fish specimen's especially genotoxic effect. But the cause of morphological and nuclear deformities is unknown and nuclear abnormalities were closely related to genotoxicity. It is suggested in future with an experimental *in vivo* study with metals and to recover the abnormal growth in fishes to protect the economic loss because fish is an important diet.

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Conflict of interest
As per authors no conflict of interest in the present study.

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