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ASSESSMENT OF NUCLEAR ABNORMALITIES IN THE PERIPHERAL ERYTHROCYTES OF FISH SPECIMEN OF SUNDARBANS COASTAL ZONE, WEST BENGAL, INDIA

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ABSTRACT

The nuclear abnormalities (NAs) in the peripheral erythrocytes of fish is an important measure to identify genotoxins in water. The present study was attempted to evaluate the frequencies of several NAs in the peripheral erythrocytes of fish, *Liza parsia* Ham. inhabited at estuarine water of Sundarbans coastal zone. Two study sites were selected, the Hatania-Doania river connected to Hooghly river as downstream site and 2Km upstream site. The frequencies of different NAs in the peripheral erythrocytes of test fish were studied during monsoon. The present results indicate an alarming risk of genotoxicity through the induction of NAs in the peripheral erythrocytes of fish *L. parsia* at a level of P<0.001 when compared between downstream and upstream. It is suggested that regular monitoring is needed for genotoxin(s) contamination level in the estuaries by genetic biomonitoring in all seasons, which is lacking in the estuaries of coastal zones at Sundarbans.

KEY WORDS : Micronucleus, Nuclear abnormalities, Genotoxicity, Genotoxins, Estuarine fish, *Liza parsia*

INTRODUCTION

The Sundarbans estuarine system, comprising the Southern part of the Indian portion of the Ganga-Brahmaputra delta front system into the Bay of Bengal (Seidensticker and Hai, 1983; Papa *et al.,* 2010; Chatterjee *et al.,* 2013; UNEP-WCMC, 2013; IUCN World Heritage Outlook, 2017) and the area are known as National Park, World Heritage Site and Biosphere Reserve (Chaudhuri and Choudhury, 1994; UNEP-WCMC, 2013; IUCN World Heritage Outlook, 2017). In past, it was reported that the Sundarbans coastal zone receives a large number of metals and organics from agricultural and industrial activities and these pollutants accumulate in the vital organs of aquatic organisms (Mitra and Choudhury, 1992; Mitra, 1998; Mitra and Zaman, 2015; Mitra and Zaman, 2016). The concentration of Cu, Zn, Fe, Pb, Cd, Cr, and Ni was determined in surface water of the rivers and estuary of the Sundarbans mangrove forest located in the southwest coastal region of Bangladesh and a higher concentration of metals such as Cd, Cr, Ni and Pb were obtained (Haque *et al.,* 2005; Shil *et al.,* 2017). It was also observed mercury concentration in the estuaries of the Sundarbans area, West Bengal (Bhattacharya *et al.,* 2014).

The fish, *Liza parsia* is an estuarian edible fish and found in and around the Kolkata fish markets. It is available almost in all seasons. The frequencies of nuclear abnormalities (NAs) in the peripheral erythrocytes of fish detect genotoxins in water and may have a potential risk of breeding due to mutation or genotoxicity. The evaluation of abnormal nuclear morphologies in fishes by different metals has been documented by researchers (Al-Sabti, 1994a; b; Allyon and Garcia-Vazquez, 2000; Ferraro *et al.,* 2004; Cavas *et al.,* 2005;

Cavas and Ergene-Gözükara, 2005; Talapatra and Banerjee, 2007; Zhang *et al.*, 2008; Talapatra *et al.*, 2014; Zohra *et al.*, 2014; Kousar and Javed, 2015; Sayed *et al.*, 2015; Elgendy *et al.*, 2017; Javed *et al.*, 2017; Hussain *et al.*, 2018).

Among NAs, micronucleus (MN) is caused due to clastogenic or aneugenic effects, in which whole chromosomes or part of the chromosomes participate as a small shaped extra nucleus within daughter nuclei during mitosis (Heddle *et al.*, 1991). It was reported by researchers that abnormal nuclear morphology serves as an indicator of genotoxic damage in fish (Pachecco and Santos, 1998; Bombail *et al.*, 2001). On the other hand, in the case of abnormal nucleus may be formed membrane blebbing and chromatin condensation (Murakawa *et al.*, 2001). The notch nucleated and binucleated cells are an indicator of abnormal cell division (Cavas *et al.*, 2005). Other NAs such as lobed, blebbed, retracted nuclei, dumble shaped nuclei, vacuolated nuclei, and nuclear cariolysis indicated cyto-genotoxic effects (Tolbert et al., 1992; Cavalcante *et al.*, 2008; Omar *et al.*, 2012; Hussain et al., 2018).

It was reported that heavy metals pollution in the estuarian water of Sundarbans coastal zone, Gangetic delta, West Bengal, Indian part (Mitra and Choudhury, 1992; Mitra, 1998; Haque *et al.*, 2005; Mitra and Zaman, 2015; Mitra and Zaman, 2016; Das *et al.*, 2018; Bonnail *et al.*, 2019) but the risk of genotoxicity for these metals have not been attempted earlier. The present study is an endeavor to detect a genotoxic effect in the peripheral erythrocytes of *Liza parsia* Ham. during monsoon, inhabited in the estuaries of Sundarbans coastal zone, West Bengal, India.

MATERIAL AND METHODS

Selection of study area

Two study sites were selected, the Hatania-Doania river estuary as downstream site (Latitude = 21°45′N and Longitude = 88°14′E) and 2Km upstream site (Latitude = 21°45′N and Longitude = 88°13′E). The study period was selected during the monsoon of 2019.

Fish sample collection

The fish specimen, *Liza parsia* Ham. (17-23 cm in length and 60-65 gm in weight) were selected for the genotoxicity experiment. This specimen is regularly trapped by the local fish catchers in the river. These fishes were collected just died from the local fish catchers. After collection from the above-mentioned sites as downstream as well as 2Km upstream, all the fishes were dissected, and immediately blood was drawn directly from the heart and proceeded for slide preparation.

Slide preparation and staining

For each fish, two microscopic slides were prepared. The clean slides were used and blood was smeared onto the slide with proper coding. The coded slides were air-dried for 12h and then fixed in absolute methanol for 10 min. After fixing, the same slides were stained in Giemsa (5%) stain for 10 min (Palhares and Grisolia, 2002; Talapatra and Banerjee, 2007).

Scoring of slides

In each fish, 1000 erythrocytes were counted for peripheral blood separately from upstream and downstream groups, respectively. The frequencies of micronuclei and other NAs in erythrocytes were detected under a Binocular microscope (OLYMPUS) using a 1000X oil-immersion lens. Frequencies of MN and NAs such as lobed nuclei (LN), blebbed nuclei (BLN), notch nuclei (NN), bi-nuclei (BN), dumble shaped nuclei (DSN), vacuolated nuclei (VN), retracted nuclei (RN), nuclear caryolysis (NC), and fragmented nuclei (FN) were expressed per 1000 cells.

Statistical analysis

All the data were analyzed to determine statistically significant differences between the study groups by using Student's t-test at 0.05. The statistical analysis was executed by using Microsoft Excel 365 Statistical Analysis add on Tool Pak.

RESULTS

The present results reveal on genotoxicity biomonitoring during monsoon through the induction of MN and NAs such as LN, BLN, NN, BN, DSN, VN, RN, NC, and FN in the peripheral erythrocytes of fish *Liza parsia* Ham. The frequencies (%) of MN and different NAs were increased in downstream fish species compared to the upstream (2km) site. In the case of MN frequencies (Fig 1), a significantly increased value ($P<0.001$) was observed in the fishes of downstream (1.41 ± 0.26) when compared to upstream (0.77 ± 0.07). The NAs such as LN, BLN, NN, BN, DSN, VN, RN, NC and FN values were also increased significantly ($P<0.001$) in the fishes of downstream (1.65 ± 0.32, 1.87 ± 0.22, 1.33 ± 0.41, 1.34 ± 0.29, 2.40 ± 0.17, 2.25 ± 0.25, 2.17 ± 0.24, 2.05 ± 0.36 and 1.76 ± 0.25) in comparison with upstream (078 ± 0.13, 1.11 ± 0.17, 0.25 ± 0.09, 0.36 ± 0.09, 1.74 ± 0.12, 1.50 ± 0.15, 1.37 ± 0.31, 1.23 ± 0.18 and 1.08 ± 0.07) respectively (Fig 1). The microphotographs of different types of NAs along with MN is exhibited in Fig 2.

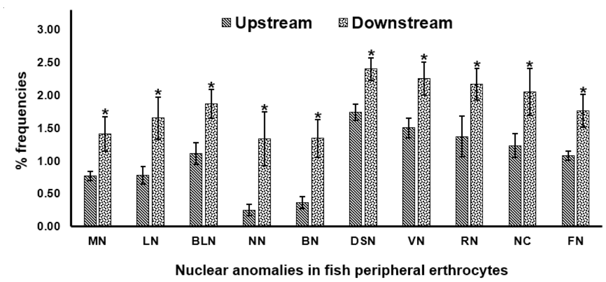

Fig. 1. Percentage frequencies of MN and NA in the peripheral erythrocytes of fish *L. parsia* (MN = Micronucleus; LN = Lobed nucleus; BLN = Blebbed nucleus; NN = Notch nucleus; BN = Bi-nucleus; DSN = Dumble shaped nucleus; VN = Vacuolated nucleus; RN = Retracted nucleus; NC = Nuclear cariolysis and FN = Fragmented nucleus; n = 10; *P<0.001)

DISCUSSION

The coastal regions of Sundarbans harbour several riverine systems. Among these, the Hatania-Doania river connects the Bay of Bengal through the Hooghly river opposite side of Sagar Island, West Bengal. The study of heavy metals in water and sediment, as well as the accumulation in the vital organs of fish, have been well documented in Hooghly river (Mitra and Choudhury, 1992; Mitra, 1998; Purkait *et al.*, 2009; Mitra and Zaman, 2015; Mitra and Zaman, 2016; Paul, 2017; Sankla *et al.*, 2018; Bonnail *et al.*, 2019). In recent research, it has been reported that several metals were accumulated in the vital organs of inhabited fish of river Ganga

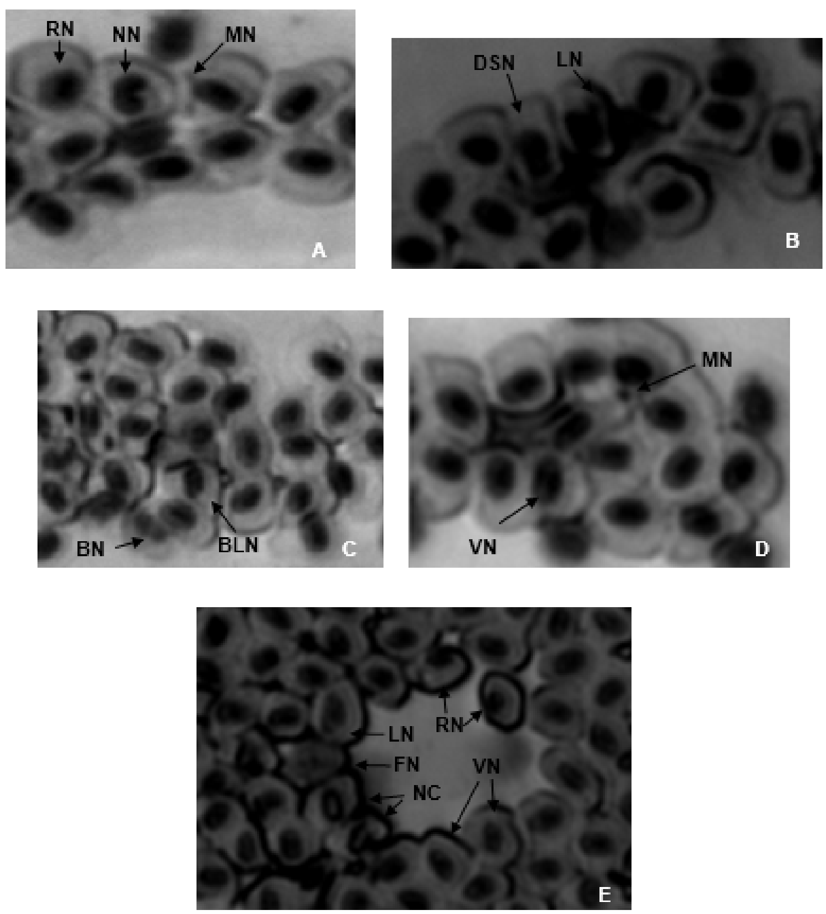

Fig. 2. Microphotographs (1000x magnification) of MN and NA in the peripheral erythrocytes of *Liza parsia* (MN = Micronucleus; BLN = Blebbed nucleus; BN = Binucleus; NN = Notch nucleus; LN = Lobed nucleus; DSN = Dumble shaped nucleus; VN = Vacuolated nucleus; RN = Retracted nucleus; NC = Nuclear cariolysis and FN = Fragmented nucleus)

(Maurya *et al.,* 2019).

This fish specimen is known as omnivorous in its feeding habits and they feed algae, diatoms, desmids, plant materials, annelids, crustaceans, bivalves, fishes, detritus, and sand grains (Bir *et al.,* 2016). The studies were observed mostly metals pollution in water and sediment, and accumulation in the organs of inhabited fish species in the coastal zone of Sundarbans. But the study is lacking for cyto-genotoxic risk especially induction of MN and NAs in the peripheral erythrocytes of this fish species due to metal exposure. However, several studies have been reported concerning the induction of MN and NAs in the peripheral erythrocytes of fish due to the presence of metals or genotoxins in the aquatic system of many countries around the globe (Al-Sabti, 1994a; b; Omar *et al.,* 2012; Hussain *et al.,* 2018).

In the present study significantly increasing trends for MN and all parameters for NAs were obtained between downstream and upstream fish species but the values were comparatively lower than others cyto-genotoxicity studies (Omar *et al.,* 2012; Hussain *et al.,* 2018). To date, the mechanism of NAs is not clearly understood but the present study may be alarming for cyto-genotoxic risk in the test fish specimen and this test model may be sensitive to genotoxin like metal or combinations of metals.

This is a first-time observational study on genotoxicity assessment with this particular test specimen, but further research is needed particularly in this area with other inhabited fish species to know the sensitivity of this test model. The alarming cyto-genotoxic risk may be threatened with edible and nutritious food like fish in the future. Generally, individual metal or combinations of metals or chemicals may alter the nuclear shape as genotoxic stress in fish (Talapatra and Banerjee, 2007; Omar *et al.,* 2012; Talapatra *et al.,* 2014; Hussain *et al.,* 2018). Moreover, the possibilities of genotoxicity may be due to the presence of metals in the river Ganga (Paul, 2017), which connects other rivers.

CONCLUSION

It is concluded that metals accumulation may lead to genotoxic risk in the peripheral erythrocytes of studied fish. Moreover, the erythrocytes are suitable cell types for genetic biomonitoring for aquatic test model and it is suggested that regular monitoring is needed for genotoxin(s) contamination level in the estuaries by genetic biomonitoring in all seasons, which is lacking in the estuaries of coastal zones at Sundarbans. In a future study, the metal analysis should be needed in the water and sediment followed by bioaccumulation in the vital organs to identify the alarming risk of genotoxicity in this test model and other fish species.

Conflict of interest

The authors declare none regarding the present study and manuscript.

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