

**Research Article**

# Diversity of Parasitic Fauna and Associated Gill Histopathology in *Cyprinus Carpio* From The Indus River, Pakistan

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[ahsan@uoswabi.edu.pk](mailto:ahsan@uoswabi.edu.pk)**Abstract**

The present study explores the diversity of parasitic infections and the associated histopathological changes in the gills of *Cyprinus carpio* (common carp) collected from the Indus River at Hund, Swabi, Pakistan. A total of sixteen fish specimens were obtained from two sampling sites and examined for parasitic infestation and gill tissue pathology using light microscopy and Hematoxylin and Eosin (H&E) staining. Multiple parasite taxa were recorded, including *Bothriocephalus acheilognathi*, *Diplostomum* spp., *Lernaea cyprinacea*, *Piscicola geometra*, *Camallanus* spp., *Chaetogaster diastrophus*, and *Nais communis*. Histopathological analysis revealed pronounced gill lesions, characterized by epithelial hyperplasia, lamellar fusion, edema, necrosis, and leukocytic infiltration, suggesting compromised respiratory efficiency associated with parasitic burden. The results highlight the relevance of parasitic infections as bioindicators of environmental stress and fish health. This study enhances current knowledge of host–parasite interactions and provides baseline information for monitoring aquatic ecosystem health and supporting sustainable fisheries management in the Indus River basin.

**Keywords:** *Cyprinus carpio*, Gills parasite, *Camallanus* spp, Hund River, Histopathology, Hematoxylin and Eosin.

## 1. Introduction

The common carp (*Cyprinus carpio*), a freshwater cyprinid, is widely distributed in rivers, lakes, and ponds worldwide and was introduced to Pakistan in 1964 for aquaculture [1]. Its rapid growth, early maturity, omnivorous diet, high fecundity, and tolerance to diverse conditions have made it a major food fish, with global production exceeding 3 million metric tons [2-4]. Often called an “ecological engineer,” it can modify habitats and expand quickly, but is vulnerable to parasites that may impair growth and reproduction [5, 6].

Fish parasites include unicellular microparasites, mainly protozoans like *microsporidians* and *myxozoans*, and multicellular macroparasites such as *helminths* and *arthropods* [7, 8]. Freshwater fishes harbor diverse ecto- and endoparasites that can impair health, growth, and survival. Common protozoans, including *Ichthyophthirius multifiliis* (white spot disease) and *Chilodonella* spp., cause gill and skin damage, excess mucus, and high mortality under heavy infestations [9]. Parasitic impacts are often worsened by environmental stressors, such as heavy metals, hydrocarbons, organic pollution, high host density, and parasite life-cycle dynamics.

Fish gills are critical multifunctional organs involved in respiration, osmoregulation, nitrogenous waste excretion, acid–base balance, and immune defense [10-12]. Comprising gill arches with filaments and secondary lamellae, they provide a highly vascularized surface for efficient gas exchange [13]. Structurally, gills consist of gill arches bearing primary filaments and secondary lamellae, forming a highly

vascularized and specialized surface that maximizes diffusion efficiency [13, 9]. Their thin epithelium and direct water exposure make them highly sensitive to pollutants, parasites, and environmental stressors, with histopathological changes such as epithelial hyperplasia, lamellar fusion, edema, necrosis, and inflammation serving as early biomarkers of compromised health and water quality [14-20]. Additionally, the presence of gill-associated lymphoid tissue (GIALT) highlights the important immunological role of gills in mucosal defense, pathogen recognition, and immune regulation [9].

Pakistan is traversed by the Indus River system, one of the largest in the world, supporting extensive irrigation networks and diverse aquatic life. The Indus River flows through Swabi District in Khyber Pakhtunkhwa, where the Hund stretch exhibits temperatures of 15–25 °C, pH 7.5–8.5, and moderate to high turbidity, providing suitable habitat for commercially important species such as the common carp (*Cyprinus carpio*). This species thrives in slow-moving waters with sandy or muddy substrates, attains lengths of 30–60 cm and weights up to 10 kg, and serves as a vital protein source for local communities. However, increasing pressures from pollution, habitat degradation, overfishing, and climate change threaten its populations, underscoring the need for conservation and health assessment through tools such as gill histopathology to support sustainable fisheries and ecosystem management.

The present study aimed to investigate gill health in common carp (*Cyprinus carpio*) from the Hund area of the Indus River. Specifically, gill samples were collected to detect parasitic infections and identify parasite types using microscopy. In addition, histopathological alterations induced by these parasites were examined through light microscopy. Finally, the results were compared with existing literature on fish parasites and gill pathology to provide a comprehensive understanding of host–parasite interactions and their impact on fish health.

## 2. Materials and Methods

### 2.1. Study area and fish sampling

The present study was conducted in the Indus River located in Khyber Pakhtunkhwa, Pakistan. The Indus River, extending approximately 3,180 km, is one of the largest freshwater systems in South Asia and supports diverse aquatic fauna, including economically important fish species. The research was specifically carried out in the Hund area of district Swabi, situated approximately 100 km northwest of Islamabad. This area was selected due to its active fishing activities, ecological variability, and reported occurrence of fish parasitic infections, making it suitable for studying gill parasites and associated histopathological alterations.

Fish specimens were collected through net from two designated sampling locations within the Hund stretch of the Indus River: Site A (Museum Site) and Site B (Motorway Bridge), as illustrated in figure 1. These sites were selected to

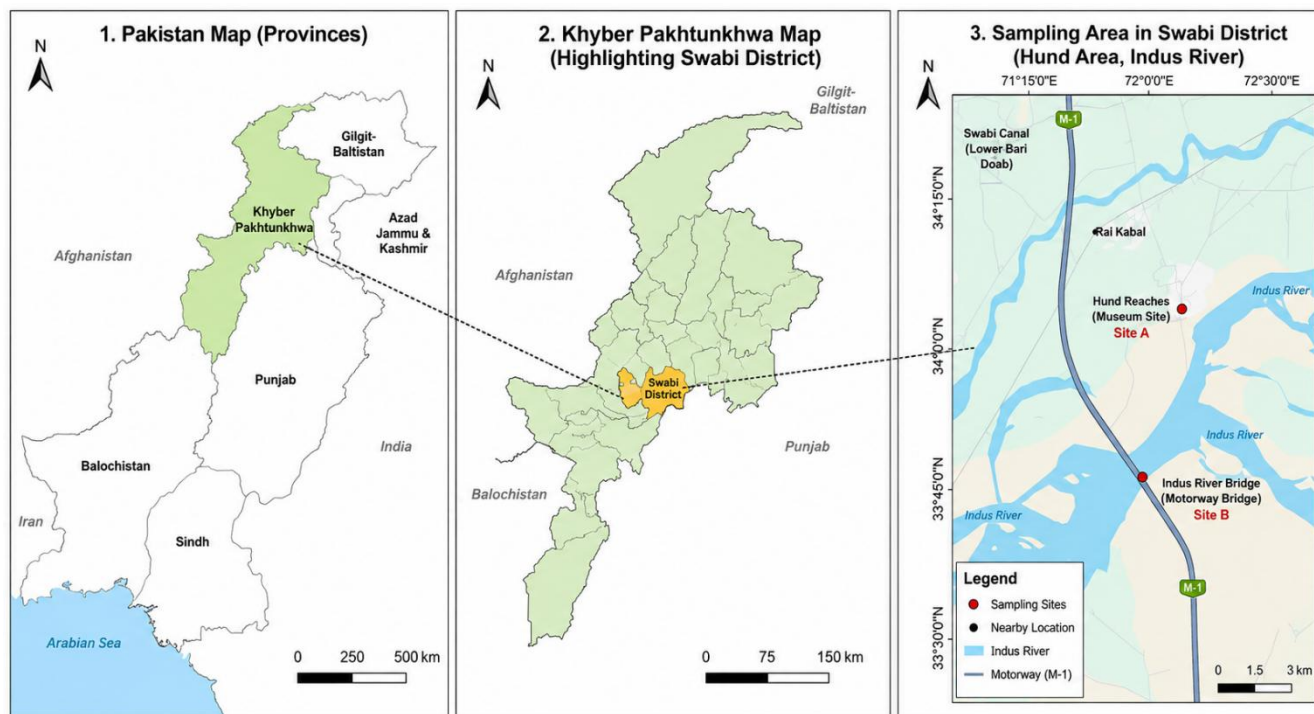
represent slightly different environmental and hydrological conditions, including variations in water flow, human activity, and habitat characteristics. During the sampling period, water temperature ranged between 15°C and 25°C, pH ranged from 7.5 to 8.5, and turbidity levels were moderate to high due to seasonal fluctuations and sediment load.

### 2.2. Parasite Identification Methodology

The gills of each collected fish were carefully examined for parasites using a stereomicroscope. Visible ectoparasites, such as copepods, leeches, and anchor worms, were gently removed with fine forceps. Gill tissues were also inspected for smaller protozoan parasites. For the detection of internal parasites, gill tissues were teased in physiological saline solution. The washed material was then placed on clean glass slides and examined under a light microscope.

Parasite identification was carried out using light microscopy based on morphological characteristics. Standard taxonomic keys and published descriptions were followed for accurate identification [8]. Parasites were classified into major groups, including protozoans, monogeneans, trematodes, cestodes, nematodes, and crustaceans. Each parasite was examined for distinguishing features such as body shape, attachment organs, and structural characteristics.

The prevalence, intensity, and abundance of parasites were recorded for each fish specimen. These parameters were used to evaluate the parasitic load and diversity in the gills of *Cyprinus carpio*. The data were further compared between sampling sites to assess variations in parasite distribution.



Sites: Site A (Museum Site) and Site B (Motorway Bridge)

**Figure 1.** Location of the study area and sampling sites (Site A: Museum Site and Site B: Motorway Bridge) along the Indus River in Hund, District Swabi, Khyber Pakhtunkhwa, Pakistan.

### 2.3. Tissue collection and histological analysis

Tissue samples from the gills were fixed in 4% neutral buffered formalin for 12–24 hours and subsequently processed for histological analysis. The fixed tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Thin sections of 3–5  $\mu\text{m}$  were cut using a microtome, mounted on glass slides, deparaffinized with xylene, and rehydrated through decreasing concentrations of ethanol to distilled water. The sections were stained with Hematoxylin and Eosin (H&E), dehydrated, cleared, and mounted with a cover slip using mounting medium. Finally, the prepared slides were examined under a light microscope to assess histological features, including epithelial lifting, hyperplasia, lamellar fusion, necrosis, and inflammatory responses.

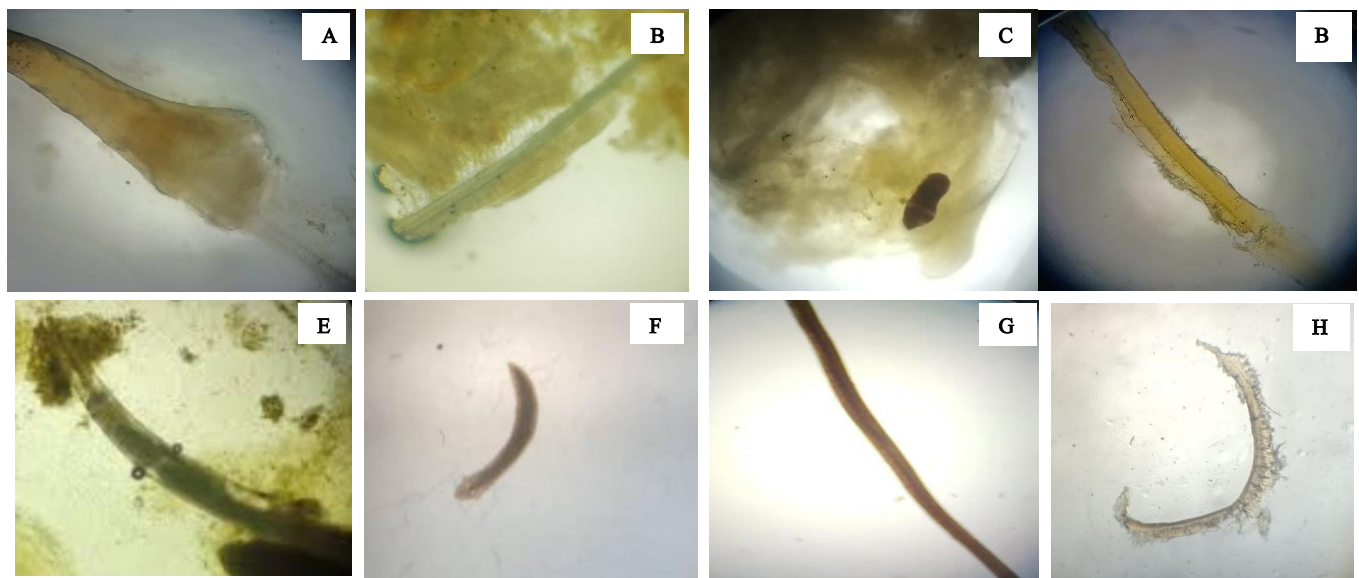
## 3. Results and Discussion

### 3.1. Diversity and percent relative abundance of order Orthopter

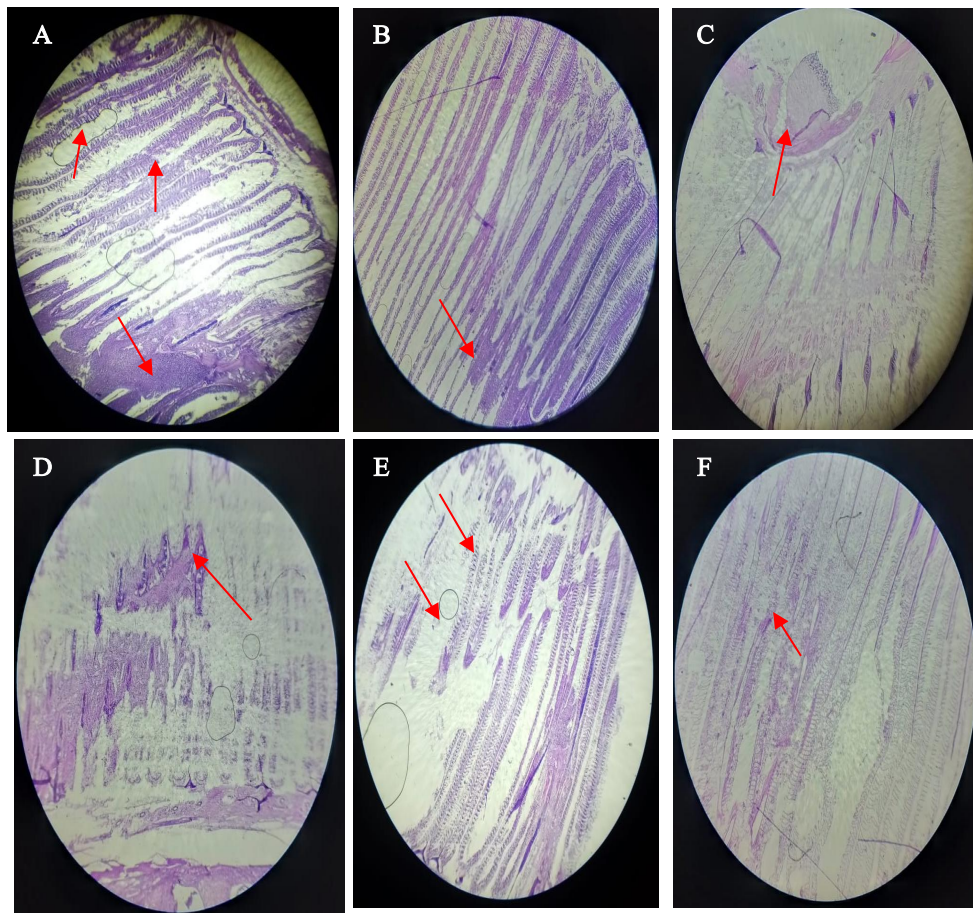
A total of 16 common carp (*Cyprinus carpio*) samples were collected from two sites in the Hund area of the Indus River, with parasites detected in six samples across both sites show in table 1. Identified parasites included helminth larvae (*Platyhelminthes*), tapeworm (*Bothriocephalus acheilognathi*), flatworm larvae (*Diplostomum* spp.), aquatic oligochaete (*Chaetogaster diastrophus*), anchor worm (*Lernaea cyprinacea*), fish leech (*Piscicola geometra*), small aquatic worm (*Nais communis*), and roundworm nematode (*Camallanus* spp.) as represented in figure 2.

Table 1. Parasite identification results from two sampling sites (A and B).

Sample No.	Site A (Museum Site) – Infection Status	Parasites Identified (Site A)	Site B (Motorway Bridge) – Infection Status	Parasites Identified (Site B)
1	Infected	Helminth larvae ( <i>Platyhelminthes</i> )	Infected	<i>Bothriocephalus acheilognathi</i> , <i>Diplostomum</i> spp.
2	Not infected	—	Not infected	—
3	Infected	<i>Chaetogaster diastrophus</i>	Infected	<i>Lernaea cyprinacea</i>
4	Infected	<i>Piscicola geometra</i> , <i>Nais communis</i>	Infected	<i>Camallanus</i> spp.
5	Not infected	—	Not infected	—
6	Not infected	—	Not infected	—
7	Not infected	—	Not infected	—
8	Not infected	—	Not infected	—



**Figure 2.** Representative helminths and aquatic invertebrates recovered during the study. (A) Platyhelminth larva. (A) Helminth larvae (*Platyhelminthes*). (B) Tapeworm (*Bothriocephalus acheilognathi*). (C) Flatworm (*Diplostomum* spp.). (D) Aquatic oligochaete (*Chaetogaster diastrophus*). (E) Anchor worm (*Lernaea cyprinacea*). (F) Fish leech (*Piscicola geometra*). (G) Small aquatic worm (*Nais communis*). (H) Round worm nematode (*Camallanus* spp.).



**Figure 3.** Histopathological alterations observed in fish gills. (A) Epithelial lifting and lamellar fusion. (B) Epithelial hyperplasia of the gill lamellae. (C) Clubbing of the gill lamellae. (D) Necrosis and edema. (E) Hemorrhage in the secondary lamellae. (F) Infiltration of inflammatory cells. Arrows indicate the observed lesions.

These parasites exhibited varied pathogenic effects on gill tissues, including mechanical damage, irritation, inflammatory responses, hyperplasia, lamellar fusion, edema, hemorrhage, and obstruction of water flow, ultimately compromising respiratory efficiency. Helminth larvae and tapeworm larvae were particularly invasive, causing tissue destruction and impaired gill function. Oligochaetes such as *Chaetogaster diastrophus* and *Nais communis* caused mild irritation and mucus overproduction, while ectoparasites like anchor worms and fish leeches attached to gill surfaces, feeding on host tissues and blood, which could result in stress, anemia, and secondary infections as represented in [figure 3](#). Overall, these findings highlight the diversity of parasitic infestations in common carp gills and their potential impact on fish health and respiration.

#### 4. Discussion

The study investigated parasitic infections and related gill histopathology in *Cyprinus carpio* collected from two sites of the Indus River (Hund area). Out of 16 samples, 3 from each site were infected with diverse parasites, including helminths,

tapeworms, nematodes, and ectoparasites. H&E staining revealed epithelial hyperplasia, lamellar fusion, edema, and leukocytic infiltration, indicating chronic parasitic stress and impaired respiratory function.

Recent studies have documented a broad spectrum of freshwater fish parasites, including nematodes, cestodes, leeches, monogeneans, and parasitic crustaceans such as *Lernaea*, which predominantly infest external organs, particularly the gills, leading to significant histopathological alterations such as epithelial hyperplasia, lamellar fusion, and inflammatory responses [21, 22]. In agreement with these findings, the present study recorded the presence of tapeworm larvae, roundworm nematodes, anchor worms, fish leeches, and flatworm larvae in the gills of *Cyprinus carpio*, accompanied by comparable histopathological changes including hyperplasia and lamellar fusion. However, in contrast to recent reports that frequently document protozoan infections in freshwater fish [23, 24] protozoan parasites were not detected in the current investigation. This discrepancy may be attributed to variations in environmental conditions, seasonal dynamics, host immunity, or differences in sampling and diagnostic techniques.

Recent studies have emphasized the importance of parasites particularly cestodes, nematodes, trematodes, and acanthocephalans as reliable indicators of environmental conditions and aquatic ecosystem health, with endoparasites considered more effective reflectors of pollution levels and ecological stress compared to ectoparasites [25, 26]. These findings are consistent with modern approaches in aquatic biomonitoring, where parasite assemblages are increasingly used to assess environmental quality [27]. In the present study, parasites such as tapeworm larvae, roundworm nematodes, flatworm larvae, and anchor worms were detected in the gills of *Cyprinus carpio* and were associated with pronounced histopathological alterations, including lamellar fusion and epithelial hyperplasia. However, acanthocephalans and protozoan parasites, commonly reported in recent literature, were not observed in the current investigation [28, 29]. This partial agreement may be attributed to differences in ecological conditions, sampling strategies, seasonal variation, host specificity, or diagnostic approaches employed in the present study.

Recent studies have reported that parasitic infections can cause significant mechanical damage, such as gill lamellar fusion, and induce physiological stress through cellular proliferation, immune modulation, and inflammatory responses, ultimately affecting fish survival, particularly under conditions of heavy infestation or environmental stress [21, 22, 25]. In the present study on *Cyprinus carpio*, gills were heavily infested with tapeworm larvae, nematodes, flatworm larvae, anchor worms, fish leeches, and *Chaetogaster diastrophus*, leading to pronounced histopathological alterations including epithelial hyperplasia, lamellar fusion, edema, and inflammatory cell infiltration. These findings are consistent with recent reports describing similar pathological responses in parasitized fish gills under environmental stress conditions [29]. The strong correspondence between the observed lesions and those documented in contemporary literature indicates that increased parasite load is closely associated with the severity of gill tissue damage, thereby reinforcing the role of parasitic infections as key determinants of fish health and survival.

Figueiredo Fernandes et al. [30], examined the effects of waterborne copper exposure on the gills and liver of Nile tilapia and reported marked gill alterations, including edema, epithelial lifting, vasodilatation, lamellar fusion, and occasional aneurysms, along with hepatic vacuolation and necrosis at copper concentrations of 1.0–2.5 mg/L over a 21-day exposure period. In the present study on *Cyprinus carpio*, gill histopathology revealed edema, epithelial hyperplasia, lamellar fusion, and inflammatory cell infiltration associated with parasitic infestation. These lesions closely resemble those induced by copper stress, particularly epithelial thickening and lamellar fusion, although the underlying stressor in the current study was biological rather than chemical. Thus, while both studies demonstrate similar gill pathology patterns in response to environmental stress, the present investigation differs in that liver pathology was not assessed and tissue damage resulted from parasitic infection rather than metal contamination.

Maftuch et al. [31] investigated *Myxobolus* sp. infection in koi carp and reported severe pathological alterations across multiple organs, including gill congestion and edema, muscle necrosis, intestinal necrosis with cyst formation, kidney necrosis with hyaline degeneration, and hepatic necrosis accompanied by inflammation. In the present study on *Cyprinus carpio*, gill tissues exhibited marked edema, epithelial hyperplasia, lamellar fusion, and inflammatory cell infiltration as a result of parasitic infestation. Although other organs were not examined, the gill lesions observed particularly edema and lamellar fusion closely correspond to those reported by Maftuch et al. [31]. This strong similarity in gill pathology suggests a consistent pattern of structural damage induced by parasitic stress.

## 5. Conclusion

This study elucidates the diversity of gill parasites infecting *Cyprinus carpio* from the Indus River (Hund area) and highlights the associated histopathological damage. Morphological and microscopic analyses revealed multiple parasitic taxa, indicating a considerable parasitic burden on this ecologically and economically important fish species. The presence of diverse parasites reflects deteriorating environmental conditions and increasing biological stress within the freshwater ecosystem.

Histopathological alterations, including epithelial hyperplasia, lamellar fusion, necrosis, and inflammatory cell infiltration, demonstrate severe gill damage that can impair respiration, osmoregulation, and overall fish health. These pathological changes suggest that parasitic infections, together with environmental stressors such as water pollution, poor water quality, and habitat disturbance, may significantly affect fish survival and productivity in the Indus River ecosystem. Therefore, regular monitoring of fish health and water quality is essential to maintain ecological balance and prevent the spread of parasitic diseases.

Overall, this study contributes to the limited parasitological and histopathological data available from Pakistan and provides a baseline for future ecological and epidemiological research. Understanding host–parasite interactions and ecosystem-related stress factors will support sustainable fisheries management, improve aquaculture disease control strategies, and help conserve native fish populations in the Indus River system.

## Author contributions

Ahsan Khan: Conceptualization, Validation, Writing-original draft, Writing -review & editing.. Areeba Aziz.: Conceptualization, Writing-review & editing. Asma: Conceptualization, Supervision, Writing -review & editing. Hafsa Iqbal: Investigation, Data curation, Formal analysis, Writing-original draft.: Writing -review & editing : Formal analysis, Validation, Writing-review & editing. All authors have read and agreed to the published version of the manuscript.

## Ethical approval

Not applicable

## Conflicts of Interest

All authors declare that they have no conflicts of interest.

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## Data availability statement

The data presented in this study are available on request from the corresponding author.

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