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Distribution Pattern of Main Mucus Secretory Cells in Different Parts of Epiderm in Epinephelus coioides

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Abstract --- Fish skin is, in fact, its first body defense line. The general skin structure is roughly similar in all fishes. It consists of two main layers: epiderm that covers the outer surface of a fish body, and its sub-layer called hypoderm that forms the inner layer. In this research, a number of 25 healthy mature Epinephelus coioides fish, with the same size and weight, from both genera were studied. For microscopic studies, cuttings from different skin areas of the fish including its head, upper lip, lower lip, dorsal and ventral trunk and tail fin, 6 µm in thickness each, were provided and stained through PAS and H & E methods. The distribution of mucus cells was studied on a scale of 100 µm^2 in epiderm. It was observed that mucus cells mainly lie in the middle to outer layers of epiderm and their distributions differ in various skin areas of the fish. Moreover, cytoplasm in mucus cells shows a positive reaction to PAS staining; therefore, they are differentiated from other epiderm cells in this way. Histometric studies illustrated that distributions of mucus cells in varying parts have significant difference ($p \leq 0.05$). However, they were not significantly different in the areas such as upper lip, lower lip dorsal and ventral trunk. According to the distribution pattern of mucus cells, the skin in Epinephelus coioides may be classified into four areas such as head, upper lip and lower lip, dorsal trunk as well as ventral trunk and tail stem.

Keywords **-- skin; *Epinephelus coioides* fish; main mucus secretory cell**

I. Introduction

Grouper fish belong to the family Serranidae and the subfamily Epinephlidea. Throughout the world, 15 genera and 159 species have been identified, known as gropers or serranidae or Seabass and Rockcods. *Epinephelus* is the main genus of this family in the Persian Gulf and the Oman Sea. Mucus secretory cells are in all areas of the epiderm. However, their frequency is variable (Blazer *et al.*, 2007; Sivakumar, 2000). The main mucus secretory cells, also known as cup-shaped cells, are mostly found in the middle to outer layers of the epiderm. However, in very thin epiderm, these cells may be located on the basal membrane (Stoskopf, 1993; Zuchelkowski *et al*., 2005). Other epiderm cells include free migratory cells, lymphocytes, macrophage, and a type of funnel-shaped cells observed in some species (McKim and Lien, 2001, Cinar *et al*., 2007). In mature fish, epiderm is a stratified squamous epithelium covering all the body surface, tail, and fins (Roberts, 2001; Campinho, 2007; Blazer *et al*., 2007). Unlike mammals, this is a living layer in fishes and is metabolically active (Guellec *et al.*, 2004; Arellano *et al*., 2004).

The shape, size, and frequency of the main mucus secretory cells greatly differ in various fish species, and even in different skin areas of the same fish (Sawsan *et al.*, 2010; Mittal, 1997). Cup-shaped cells are larger than epithelial ones, and positively react to histochemical staining such as PAS and AB. Due to the frequency of fish species and considerable differences in their tissues and organs, anatomical and histological studies of certain species cannot be generalized to others. Also, skin samples are used for the diagnosis of many diseases in cytological experiments. However, there is no report on the natural skin structure of *Epinephelus coioides* and the distribution of mucus cells. Therefore, the present study is conducted to determine the histomorphology, histometry, and histochemistry of the skin in different body areas of the fish.

II. Materials and methods

In this research, 25 *Epinephelus coioides* fish from both genera, with a mean weight of 550 ±54 gr and a mean length of 29.25 ±3.25 cm, were provided from the research station in the Persian Gulf. Following their sampling, they were placed in boen solution and transferred to the Aquatic Histology Laboratory in Khorramshahr University of Marine Sciences and Technology. For microscopic study of the main mucus secretory cells, a number of 6µm-tick cuttings from different skin areas of *Epinephelus coioides* including its head, upper lip, lower lip, upper trunk, lower trunk, and tail stem were prepared by means of a rotary microtome (Model Leica RM 2245) and stained through periodic acid Schiff (PAS) as well as hematoxcilin and eosin (H & E) methods (McKim *et al.*, 2004; Kristy and Weir Lunan, 2008).

Moreover, for histometric studies on the distribution of the main mucus secretory cells, at least six cuttings were provided from each sample. Then, in each cutting, a scale of 100 µm^2 of the skin epiderm of the fish was studied from five microscopic sight fields through a Nikon light microscope (Model Eclipse E200) equipped with Dinolite lens. Then, by means of SPSS Software (ver. 16) and the help of variance analysis test, the histometric results were studied concerning the similarity of variability in different epiderm areas of the skin in *Epinephelus coioides*, and after Tukey test, they were analyzed to compare the epiderm of different skin areas of each two parts, and also to determine the significance or insignificance of their difference.

III. Results

The histological results of this research showed that, in *Epinephelus coioides*, there are spherical cells in its skin epiderm located between epithelial cells and in the middle to outer layers of the skin epiderm. These cells are situated as groups in some adjacent rows and their cytoplasm took a cherry red color in the H&E specific staining due to their structure (*See* Fig. A).In PAS staining, these cells have a quite clear foamy cytoplasm and are characterized as the main mucus secretory or cup-shaped cells (*See* Fig. B). The cell nuclei were as extended heterochromatins located at their bases.

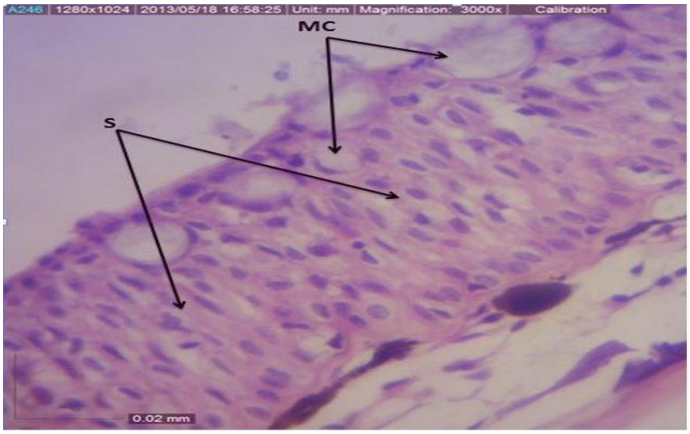

Fig. A. Distribution of mucus cells and stratified squamous epithelium of epiderm in lower trunk in H&E staining (H&E, ×300)

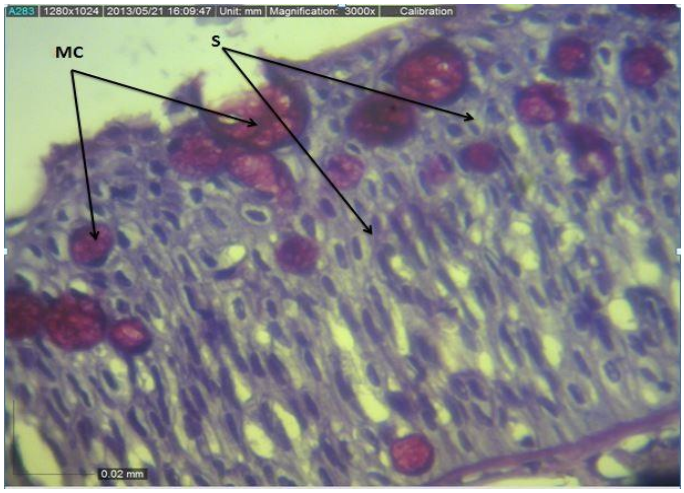

Fig. B. Distribution of mucus cells and squamous cells in the epiderm of tail stem in PAS staining (PAS ×3000)

The distribution of mucus cells in head, at $p \leq 0.05$ level, was higher than other parts of the epiderm in *Epinephelus coioides* (*See* Fig. C), and its lip epiderm had the second highest number of mucus cells (*See* Fig. D).

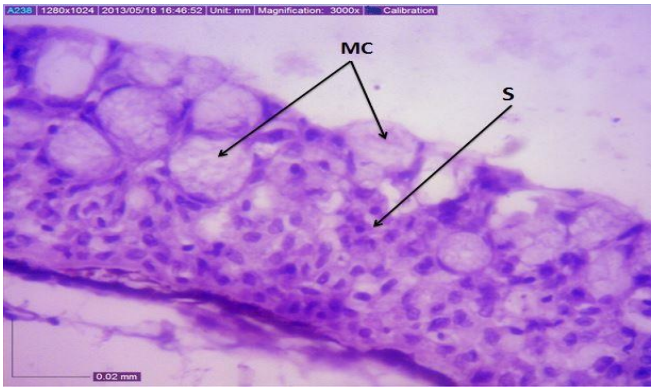

Fig. C. Distribution of mucus cells in the epiderm of head in *Epinephelus coioides* in H & E staining (H & E, ×3000)

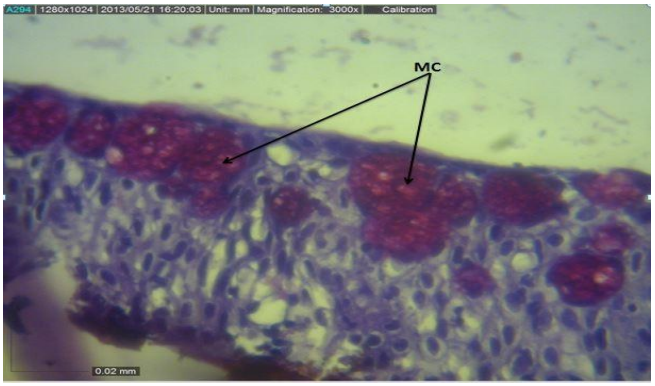

Fig. D. Distribution of mucus cells in the epiderm of lips in *Epinephelus coioides* PAS staining (PAS ×3000)

In addition to mucus cells, in middle and deep layers of most areas of skin epiderm in *Epinephelus coioides* except its lips, there were cells located among epiderm epithelial cells that negatively reacted to PAS specific staining and were observed as heterochromatin nuclei in H & E staining. These cells had a clear eosinophilic cytoplasm and, in some of them, vacuoles were also observed (*See* Fig. E and Fig. F)

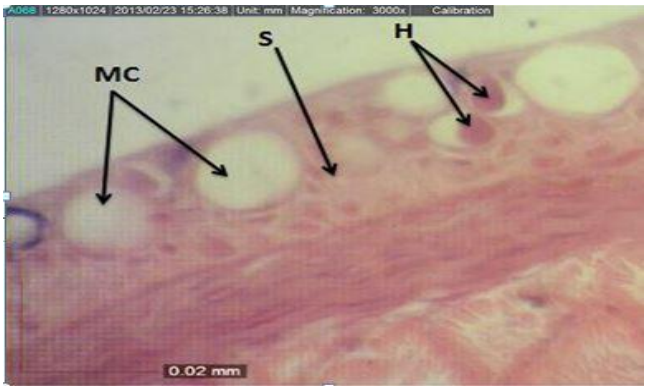

Fig. E. Distribution of warning cells and mucus cells in the epiderm of upper trunk in H & E ×300)

Fig. F. Distribution of warning cells in the epiderm of head in PAS staining (PAS ×300)

As shown in Table 1 and Table 2, histometric results of the research also illustrate that different skin areas in *Epinephelus coioides* have significant differences regarding the number of main mucus secretory cells ($p \leq 0.05$).

Table 1. Mean and standard deviation (SD) of mucus cells on a scale of 0.02 µm² of epiderm in different skin areas in *Epinephelus coioides*

Position	Head	Upper lip	Lower lip	Upper trunk	Lower trunk	Tail stem
Distribution of mucus cells	85±2.19	40.8±1.92	40.4±2.50	28.6±2.07	15.8±2.38	15.4±1.14

Table 2. Significance values and double comparison of the distribution of mucus cells in different skin areas in *Epinephelus coioides* on a scale of 100 µm²

Position	Head	Upper lip	Lower lip	Upper trunk	Lower trunk	Tail stem
Head	-	0.000*	0.000*	0.000*	0.000*	0.000*
Upper lip	0.000*	-	1.000	0.000*	0.000*	0.000*
Lower lip	0.000*	1.000	-	0.000*	0.000*	0.000*
Upper trunk	0.000*	0.000*	0.000*	-	0.000*	0.000*
Lower trunk	0.000*	0.000*	0.000*	0.000*	-	1.000
Tail stem	0.000*	0.000*	0.000*	0.000*	1.000	-

*: There is a significant difference at 0.05 level.

The statistical data from Table 1, Table 2, Diagram 3, and double comparison of different skin areas indicate that the number of main mucus secretory cells did not show a significant difference just in upper lip (40.8±1.92) and lower lip (40.4±2.50). Moreover, lower trunk (15.8 ±2.38) and tail stem (15.4±1.14) had no significant difference. There were, however, significant differences between other parts.

According to the results, we can divide the skin of *Epinephelus coioides*, regarding the number and distribution of main mucus secretory cells, into four areas respectively as follows:

1. *Head area skin* where the distribution of main mucus secretory cells was 85.2±2.19 ones in 100 µm² of epiderm length and had the highest number of mucus cells.

2. *Lip area skin* that is divided into upper lip and lower lip. The distributions of mucus cells in upper and lower lips in 100 µm of epiderm length were 40.8±2.50, respectively.

3. *Upper trunk area skin* where the distribution of mucus cells was 28.6±2.07 ones in 100 µm of epiderm length.

4. *Tail stem and lower trunk area skin* where the distributions of mucus cells in 100 µm of epiderm length where 15.4±1.14 and 15.8±2.38, respectively. Moreover, they had the lowest number of mucus cells.

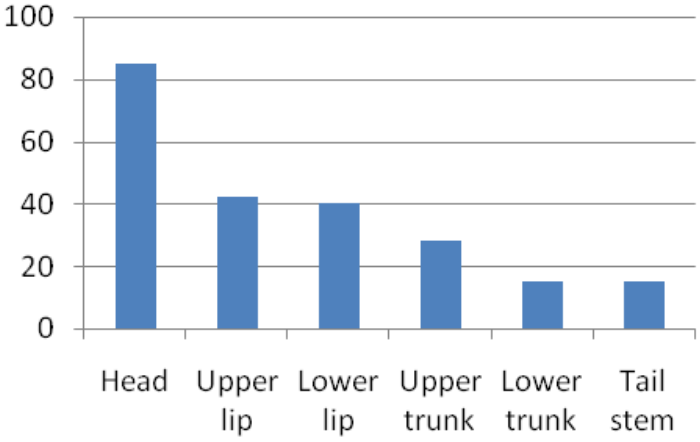

Diagram 3. Comparison of mean number of main mucus secretory cells in 100 µm of skin in *Epinephelus coioides*

IV. Discussion

The results of histological and histochemical studies on skin epiderm in *Epinephelus coioides* showed that, in addition to epithelial cells, there are two types of secretory cells, which are much larger than epithelial ones in size. The first group is the cells that positively reacted to PAS staining. These cells, which are the main mucus secretory ones, are responsible for the production and secretion of mucus and cover the body surface. Since a major part of their secretions is of mucopolysaccharide and glycoprotein types, they have positive reactions to PAS staining. However, the second group included the cells that negatively reacted to PAS staining and their cytoplasm took no color. These cells, characterized as those secreting the warning substances, are club-shaped. As these club-shaped cells mainly contain protein, they showed negative reaction to PAS staining.

The results of the present study on *Epinephelus coioides* were consistent with those obtained by (Stoskopf, 1993). Which also suggested that there are no warning cells in its lips. The main mucus secretory cells, also known as cup-shaped cells, are mostly witnessed in middle to outer layers of epiderm. However, in very thin epiderm, these cells may be located on the basal membrane (Stoskopf, 1993; Ghattas and Yani, 2010). In the existing research, the main cells were also observed in upper and lower layers of epiderm. The number of mucus secretory cells in head and trunk areas was more than other body parts. They were

located in the more middle epiderm layers and were usually spherical. When migrating towards epiderm surface, these cells became matured, wide, extended, and massive and their size increased. When mucous secretions in these cells are usually complete and available, the nuclei and organelles move to their bases. As these cells reach epiderm surface, the membranes tear in the apical point and their contents are released (Shepherd, 1994; Roberts, 2001; McKim, *et al*., 2001; Stoskopf, 1993).

The results were consistent with those of the present research, suggesting that head area epiderm had the maximum distribution of mucus cells, while stem and lower trunk having the minimum distribution of them. According to Whiter *et al*. (1986), cup-shaped cells are divided into two general groups including cup-shaped mucus cells and cup-shaped serous cells. The vesicles in the cup-shaped mucus cells contain mucous glycoproteins that mostly took faint basophilic (blue) or cherry color in common histological sections. However, the vesicles of the cup-shaped serous cells contain general proteins and often take acidophilic (red) color in common histological sections.

The results of the histometric studies in the present research showed that there is a significant difference between various skin areas in *Epinephelus coioides* concerning the number of main mucus secretory cells. The highest number of these cells was observed in lips, head, and upper trunk. Given the role of these cells, it may be an adaptive factor due to the lack of scale in these areas. In other words, the large number of these cells causes the development of a thicker mucus layer and, as a result, provides a better protection of the skin. The main mucus secretory cells in some fishes aggregate as groups in different body areas and formulate multicellular holocrine glands (Mittal, 1997; Park *et al*., 2000). Also, in the existing research, we witnessed such cell aggregations, and they were clearly observed in the skin epiderm of different areas.

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