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Seasonal histological roles of M₂ macrophages in kidney carp fish *Cyprinus carpio* L

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Abstract

The study was carried out on forty adult male carp fish during different months all over the year. Samples were obtained from cages for fish rearing from Dijlah river in Baghdad during 2017. After sacrificing the fish, specimens were prepared for light microscopy. The study revealed that Carp fish had relatively small macrophages. Different sizes of macrophages were identified. The number and size of macrophages varied with seasons. The size of macrophage increases with the activity of season. Kidney tissue repair was regenerated by interstitial tissue and renal epithelial cells. Macrophages and basement membrane play an important roles in regeneration. Macrophages migrate to the damaged tissue in serial pattern along with the basement membrane, then distributed randomly among the interstitial cells of area of tissue reaction. During movement, the nucleus of macrophage lies cranially. Macrophages tend to unite with each other rather than to divide. Binucleated macrophage was derived from the union of two smaller ones and not from nuclear division. The nuclei of giant cell lies peripherally. The presence of macrophages in the interstitial tissue was lasted all over the year. Five stages of macrophage migration and five steps of macrophages union were firstly hypothesized and described.

Keywords: Fish, Histology, season, giant cell, Macrophage, basement membrane

1. Introduction

Two types of macrophage were present i.e., M₁ macrophage that elicit inflammation, with nuclei dispersed throughout their cytoplasm and the M₂ macrophage that stimulate tissue repair, with their nuclei dispersed at the periphery^[1]. Macrophages are found near the connective tissue capsule and in the perivascular regions. They are present mostly as a single cell, or in small groups. The diameter of human macrophage was about 21 micrometers^[2]. They are large, irregular with branched processes. Their cytoplasm is granular and vacuolated with irregular eccentrically located nucleus. They are fixed or mobile and may unite with each other to form the large giant cell with several nuclei^[1, 3]. The macrophages classified on basis of function into activated macrophages, wound-healing macrophages, and regulatory macrophages^[4]. The functions of macrophage are not yet certain. They also have an important role in removing cell debris and damaged components formed during the physiological involution process. They are also secretory cells producing chemo tactic factors, enzymes (collagenase) and cytokines that participate in defensive and reparative functions. Macrophage may survive for months in the tissues. A typical macrophage in man measures between 10-30 um in diameter^[2, 5], Kumar *et al.* 2016^[6] reported the increased of melanin in fish, especially in stimulating season of lay eggs in post spawning phase and decreased in resting season. Couillard and Hodson (1996)^[7] stated that the density of macrophages used as a marker of toxicity. Minton 2016^[8] stated that the chronic inflammatory stimuli promote the immune cell aggregation. On the other hand, basement membrane influence cell metabolism, regulate cell proliferation and differentiation by binding and concentrating growth factors, and serve as pathways for cell migration^[2, 9], This study focuses the light on many aspects of macrophages especially their migration and the process of giant cell formation so as to develop better understanding of their phenotype cellular biology.

2. Materials and methods

Forty adult apparently healthy male Carp fish *Cyprinus carpio* L were obtained from cages reared at the edges of Dijlah river in Baghdad in 2017. Samples were collected in four seasons all over the year. Specimens of kidneys were collected immediately after sacrificing. Samples were fixed with 10% Neutral buffered formalin, processed by routine histological techniques

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for light microscopy. Hematoxylin and eosin (H&E) stain and periodic acid-Schiff (PAS) stains were used. Oculometer was used for measurement ^[10].

3. Results

Carp fish had relatively small macrophages with a diameter of (2.3-8.8) μm^2 (Table 1). The study found that there were four sizes of macrophages; small, medium, large and giant cells. The size and number of macrophages varied with seasons. Small-sized macrophages were mostly increased in Autumn season, while medium and larger ones were predominated in winter and spring seasons respectively. Winter and spring seasons recorded the higher number of macrophages; autumn, and summer comes later. As macrophages directed toward the area of tissue reaction, their size was increased. During movement of macrophage, nucleus occupies the cranial part. The thickness of basement membrane increased in active seasons (Table.1). Macrophages present either individually or collectively in regular serial waves along the basement membrane (Fig. 1) or irregular clusters (Fig. 2 and 3). Kidney tissue repair was performed by interstitial cells and renal epithelial cells, with supported by the macrophage that encourage tissue repair. The present results demonstrated that macrophages were functionally present all over the year. The cytoplasm appeared brown or black with H & E stain and purple-magenta color in PAS stain (Fig 1 and 2). Seasonal environmental variations leading to damage of the tissue elicit the production of melanin within the cytoplasm (Fig. 5, 6). The study explain firstly that the process of union between two macrophages achieved when pseudopodia from the first cell engulf the nuclear-containing cytoplasmic projection of the second and then the open edges of the cytoplasm of two macrophage were united (Fig.1 and 4). On the other hand, the present study hypothesized that the migration of macrophage includes five stages, namely,

1. Stage of monocytes migration: Upon stimulation, monocytes migrate from the lumen of the blood vessels to the area of reaction as waves in series pattern, then they distributed as irregular clusters around the area of tissue reaction.
2. Stage of activation: In the tissue, monocytes modified from resting to stimulating states. Monocytes or macrophages unite with each other to form the larger giant cell.
3. Stage of tissue reaction: Macrophages arranged in groups, intermingled with the cells of interstitium, adhere to the damaged renal tubules,

4. Stage of giant cell formation which includes many steps:
 - a. Step of recognition
 - b. Step of pseudopodia formation from the first macrophage, and the nuclear-containing cytoplasmic projection from the second macrophage.
 - c. Step of adhesion of the pseudopodia with the projection.
 - d. Step of phagocytosis of the nucleus.
 - e. Step of union of the free edges of cytoplasm
5. Stage of macrophages withdrawal: Some of macrophages were noticed at the centers of area of tissue reaction, others were at the periphery, away from the center.

4. Discussion

The present study revealed that the size and number of macrophages were varied with seasons, this was confirmed with Couillard and Hodson (1996) and Kumar *et al.* 2016 ^[6, 7] who reported that macrophages were increased in active seasons. Macrophages also stimulate cells that reepithelize the wound and lay down a new extracellular matrix ^[11-13].

Smaller macrophages unite with each other to form the huge giant cell. This was confirmed with Minton 2016 ^[8] and in variance with Robbins *et al.* 2013 ^[14] who reported the possibility of macrophage to proliferate. The eccentrically located nucleus of macrophage confirms that this cell never divides. The presence of macrophage at the damaged renal tubules may explain its role in the repairing events. This was to secrete cytokines which carried out many biological processes like cellular growth, apoptosis, interstitium build up. This was confirmed by Mesher 2010 ^[2] who reported that basement membrane enhances cell proliferation and differentiation through preparing growth factors. Mesher 2010 ^[2] reported that macrophages function for several months. This was similar to the findings of the present study. The high affinity of macrophages for PAS stain indicates the high quantity of glycogen, and mucosubstances in their cytoplasm, this was in consistent with Chok kalingam *et al* 2015 ^[15]. The study was confirmed with Gomes *et al.* 2005 ^[16] who showed that the nuclear movement was the initial event in migrating cells which is governed by the microtubule-organizing center (MTOC). It was concluded that macrophage activity was affected by extreme cold or hot environment. Besides, macrophage nucleus never divide, otherwise several macrophages unite to form one giant cell. The study declared that the union of macrophages performed by endocytosis of the pre-existing macrophage to the nuclear-containing cytoplasmic projection of the new macrophage.

Table 1: Numbers and diameters of macrophages of kidney carp fish during four seasons

Seasons	Number of macrophages/mm ²			Diameter of Macrophages(μm)		Thickness of basement membrane
		Small	Medium	Large		
Winter	15.65±0.42 a	2.33±0.03 a	4.31±0.03 a	8.81±0.03 a	1.32 ± 0.02 a	
Spring	11.55±0.04 b	2.12±0.07 a	4.15±0.17 a	6.13±0.77 b	1.21 ± 0.85 b	
Summer	7.37±0.43 d	2.32±0.02 a	4.22±0.02 a	5.52±0.12 c	1.00 ± 0.93 c	
Autumn	9.77±0.98 c	2.43±0.33 a	4.13±0.37 a	4.53±0.22 c	1.02 ± 0.97 c	
P<	0.05	NS	NS	0.05	0.05	

- Numbers represent means± SE.
- Similar small letters represent no significant differences at the same column.
- Different small letters represent significant differences at level of (P<0.05) at the same column.

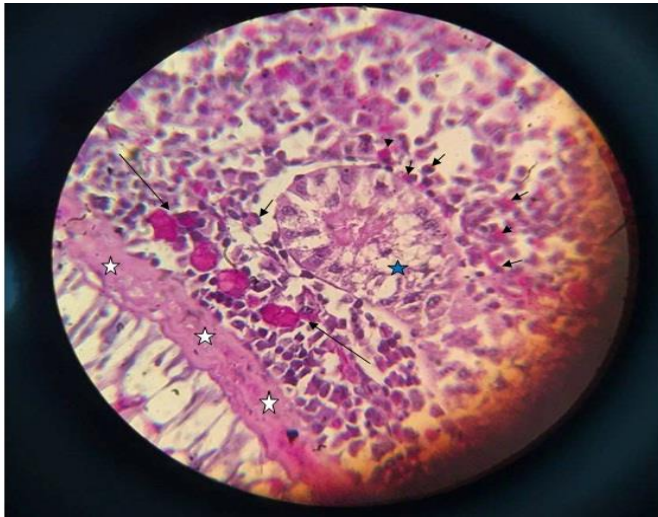


Fig 1: A wave of large non phagocytic type of macrophages in winter. Long arrows refer to the process of adhesion between macrophages to give rise to giant cell. Note small (small arrows) and medium (arrow heads) macrophages attached to damaged proximal convoluted tubule (blue star) in renal tissue of carp fish. White stars point the enlarged basement membrane. X1000. PAS stain.

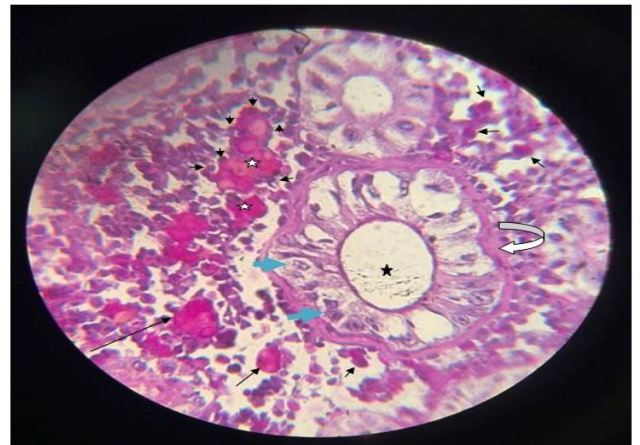


Fig 4: Carp fish kidney in winter. Small macrophage (small arrows), medium macrophage (medium arrow), and large macrophage (large arrow). white stars refer to giant cell, note the eccentric nuclei lies at the periphery (small arrows) and the process of fusion (endocytosis) between star-labeled macrophage and giant cell. Blue arrows refer to binucleated cells in renal tubular epithelial cells. Black star points to renal tubule surrounded by basement membrane (curved white arrow) X1000. PAS stain.

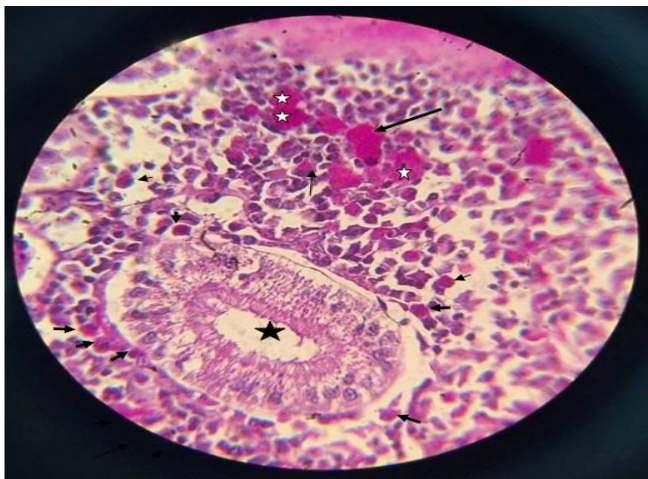


Fig 2: Different sizes of macrophages in repairing process in winter. Small arrows refer to small macrophages surrounded damaged renal tubule (black star). Medium arrow refers to medium macrophage, Long arrow indicates large macrophages. White stars point the union of macrophages. X1000. PAS stain.

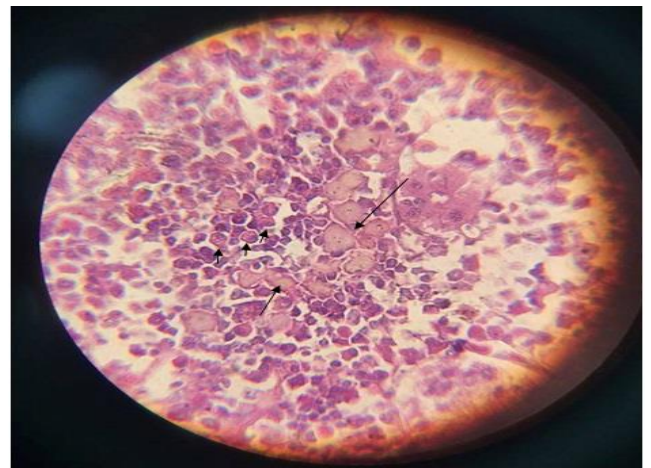


Fig 5: Different sizes laden-melanin macrophages intermingled with the interstitial tissue in Autumn. Long arrow refers to large macrophages, medium arrow refers to medium macrophage, and Small arrows indicate small inactive macrophages. Note the sizes of macrophages were smaller than spring and winter times X1000. H & E stain.



Fig 3: Kidney Carp fish in spring, macrophages accumulate in the center of the interstitium. Blue arrows refer to union of macrophages. Small arrows refer to small macrophages. x1000. PAS stain.

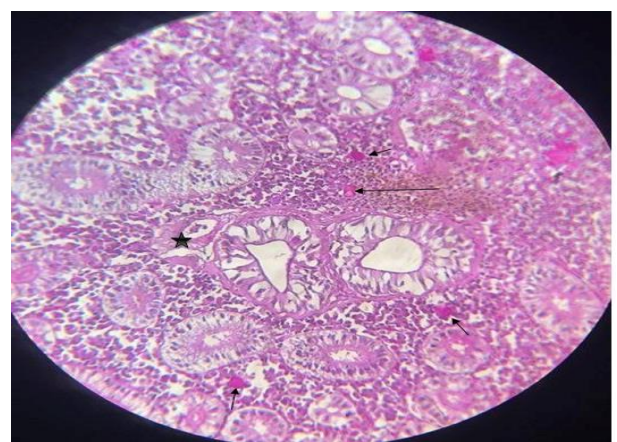


Fig 6: Kidney of Carp fish in summer. Few medium macrophages in the center and periphery of interstitial tissue (small arrows). Long arrow points to the union between two small macrophages. black star refers to blood vessel. X1000, PAS stain.

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