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Comparative Analysis of Hematopoietic Stem Cells in the Head and Trunk Kidneys of Common Carp (*Cyprinus carpio*) Using Histology, Immunohistochemistry, and Flow Cytometry

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Abstract

HEMATOPOIETIC stem cells (HSCs) serve a fundamental function in blood cell production within vertebrates. In teleosts such as common carp (Cyprinus carpio) the kidneys (notably the pronephros and mesonephros) are vital to hematopoiesis. This study aimed to investigate HSCs distribution across these specific kidney regions, employing a variety of techniques (including histology, immunohistochemistry and flow cytometry). The hematopoietic organs, which comprise head kidney (pronephros) and trunk kidney (mesonephros), underwent meticulous dissection. Various histological methods revealed the localization of hematopoietic cells; however, immunohistochemistry proved critical in highlighting regions that express CD34. Although diverse methods were employed, this comprehensive approach ultimately enhanced our understanding regarding HSC distribution within kidneys. The amalgamation of Hoechst 33342 tagging, discerned via flow cytometry, has facilitated the scrutiny of diverse cellular subpopulations and their respective stages within the cell cycle this is crucial for comprehending these processes. The common carp kidney comprises two principal regions: the head kidney, which predominantly harbors a robust population of hematopoietic cells enveloped by blood vessels and the trunk kidney, recognized for its nephrons and associated hematopoietic tissues. Both regions encompass nucleated blood cells; however, there appears to be a heightened number of cells in the G0/G1 phase within the head kidney. Hematopoietic stem cells occupy the trunk kidney (this is significant) because they exhibit the surface markers CD34 and CD41. A map indicates that HSCs are more plentiful in the trunk kidney as opposed to the head; however, this enhances our understanding of fish hematopoiesis.

Keywords: Kidney, common carp, hematopoietic stem cells, flow cytometry.

Introduction

Hematopoietic stem cells (HSCs) are the source cells for all types of blood cell [1,2,3]. In mitosis, (HSCs) undergo division to yield two distinct cells. One cell, however, evolves into a hematopoietic progenitor cell (HPC), which retains the shape, genes and function of its original stem cell. Although the differentiation occurs, this process is vital for sustaining hematopoiesis, because it ensures the continuity of cellular identity [4,5,6]. The hematopoietic progenitor cell (HPC) divides to produce daughter cells, which then proliferate into various types of blood cells; nevertheless, this process is not without difficulties. Although HPCs play a critical role in hematopoiesis, the routes via which these progenitor cells develop are still being investigated [7,8,9,10]. Hematopoietic tissue is contingent upon the provenance of the cells. However, the classification is not merely a matter of origin; this classification affects functionality and pathology. Although the distinctions among these types are significant, they often intersect. Because of this complexity, understanding the nuances of hematopoietic lineage is essential for advancing therapeutic strategies in hematological disorders [11,12].

The common carp (Cyprinus carpio), a freshwater fish, flourishes in many nutrient-rich locations throughout Europe; nonetheless, its success is dependent on certain environmental conditions. It

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thrives in slow-moving waters with dense vegetation and has extraordinary tolerance to habitat alterations (for example, low oxygen levels). This flexibility is critical because it enables the species to withstand a variety of ecological difficulties. Although the common carp is resilient, its expansion can cause ecological imbalances, which raises worries among environmentalists [13]. When confronted with pathogens (e.g., bacteria and parasites), fish necessitate robust defense mechanisms; this is particularly evident in numerous species. Blood cells play a crucial role in immune responses, demonstrating that blood cell production is essential for their continued existence. However, these systems are generally effective, they can be compromised due to environmental factors. Although survival is often contingent upon such defenses, certain species exhibit remarkable resilience, because they adapt to varying conditions [14].

The pronephros is crucial for blood cell formation in carp; this anatomical structure serves a function analogous to that found in other teleost fish [14,15]. Other organs, such as the mesonephros [16], thymus, spleen [17] and serosa of the middle intestine, may exhibit blood cell production. However, the pronephros performs numerous functions: it is involved in blood cell creation, cell storage, immune responses and hormonal regulation. Although this organ's role is multifaceted, it is crucial to recognize that the interplay among these functions can complicate the understanding of their individual contributions [18,19].

Hematopoietic stem cells are located in the kidney marrow, situated alongside the urinary tubules. Witeska et al. [20] elucidated that directed stem cells originate inside the hemogenic endothelium and might differentiate into several hematopoietic lineages. In common carp, the pronephric kidney serves as the largest organ for manufacturing pink blood cells, lymphocytes and thrombocytes; however, its significance extends well past hematopoiesis. This organ, despite the fact that essential for hematopoietic methods, additionally performs an indispensable position in various physiological capabilities. Because of this multifaceted importance, understanding the complexities of its biology is crucial for similar improvements within the area [4,21].

Numerous studies have provided quantitative insights into the proportions of different blood cell lineages found in the hematopoietic organs of teleost fish [14,20,22,23,24,25,26,27]. However, there is a scarcity of data concerning hematopoietic stem cells in the pronephros and mesonephros of common carp. Thus, this research employed histology, immunohistochemistry, and flow cytometry techniques to evaluate and map hematopoietic stem cells in the pronephros and mesonephros of Cyprinus carpio.

Material and Methods

Fish collection

Fifteen adult common carp, each weighing around 400-500 grams and including both males and females, were collected. Samples were gathered throughout various seasons from a private fish farm in the Kafer El-Sheikh governorate. The fish were then transported in water-filled boxes to the Anatomy Department at the Faculty of Veterinary Medicine, Mansoura University.

Histological and immunohistochemical analysis

Fish were euthanized in chilled water using a 0.2% tricaine solution. After confirming death, dissection was carried out with sharp scissors to remove the internal organs from the abdominal cavity. The head kidney, about 0.5 cm^3 in size, was extracted using biopsy tweezers and prepared for paraffin embedding. Serial cross and sagittal sections were cut to a thickness of 5 μm, stained with Ehrlich's hematoxylin and eosin, and examined under an Olympus light binocular microscope equipped with a Canon 4000D camera. The staining protocols followed the methods described by Suvarna et al. [28]. In the context of immunohistochemical examination of CD34 within renal tissues taken from odd carp, the deparaffinized slides were subjected to antigen retrieval using a citrate buffer before being blocked with 10% donkey serum. The slides were then treated with a rabbit anti-human CD34 monoclonal antibody (cat: A19015, ABclonal, China) diluted to 1:200. Following that, they were treated with a biotinylated donkey anti-rabbit secondary antibody (711-0. Five-152, Jackson ImmunoResearch, West Grove, PA, USA). Certain antibodies were seen by incubating in a diaminobenzidine solution (SK-4103, Vector Laboratories, Newark, California, USA) [29].

Preparation of kidney hematopoietic stem cells for flow cytometry

Extraction of hematopoietic cells

After euthanizing the fish, both the head (HK) and trunk (BK) kidneys were taken out of the celomic cavity. Hematopoietic cells were isolated by grinding the kidneys on a stainless steel mesh with forceps in 5 ml of Hank's balanced salt solution (HBSS) that was supplemented with 2% fetal bovine serum (FBS) [7]. The kidney cells obtained were then collected through centrifugation. The supernatant was discarded, and the pellet was gently mixed with 2 ml of distilled water to cause osmotic lysis of the erythrocytes. After this, an additional 2 ml of HBSS was added, and the cells were washed twice with HBSS using centrifugation.

Hoechst 33342 staining of hematopoietic cells

Kidney hematopoietic cells were stained with Hoechst 33342, the usage of a changed protocol

initially designed for mammalian bone marrow cells [30]. To identify the top of the line staining method for the hematopoietic cells of commonplace carp, we examined numerous concentrations of the H33342 solution at unique temperatures and incubation times. The cells had been prepared at an awareness of 10^6 cells/ml in Hank's Balanced Salt Solution (HBSS). We brought a very last attention of 7. Five mg/ml of H33342 to the cell suspension, which became then incubated for ninety mins at 25°C. Flow cytometry evaluation and sorting were performed with a dual-laser flow cytometer (EPICS ALTRA, Beckman Coulter) ready with 488 nm and UV argon lasers. The Hoechst dye become excited by the UV laser, and fluorescence became measured at specific wavelengths using 410/20 (Hoechst blue) and 575/20 (Hoechst crimson) band-bypass filters. Propidium iodide (PI) fluorescence was excited by way of the 488 nm laser and detected through a 675/20 band-pass filter out.

To assess the samples, a gate was set up to remove worthless cells that tested positive for propidium iodide (PI) and any unstained particles. A dot plot was used to investigate Hoechst blue and Hoechst pink fluorescence in the gated region. The aspect population (SP) cells, recognized by means of their awesome positioning, had been sorted into polypropylene tubes containing one hundred% fetal bovine serum (FBS). Gating become carried out using forward scatter (FSC) and side scatter (SSC) to categorize cells based totally on their size and granularity, with FSC usually indicating cell length and SSC reflecting the complexity or granularity of the cells.

Flow cytometry analysis of CD34 and CD41 (Flow Cytometric Immunophenotyping)

Immunophenotyping involved combining PBS-azide with fluorochrome-conjugated antibodies. Cells were analyzed on a Becton Dickinson FACScalibur with Cell Quest software. A kidney hematopoietic mononuclear cell solution was treated with Rh-123 and stored. Rh-123 accumulation and CD34/CD41 positive levels were examined using Paint-a-gate software. Statistical comparisons were made using the Friedman test and Spearman correlation [31].

Results

Gross anatomy of the kidney subdivisions in common carp

The kidneys of common carp appeared as slender-shaped, elongated organs with a dark reddish-brown coloration located on the roof of the abdominal cavity, just ventral to the vertebral column (Fig. 1). They were split into two halves (right and left) based on their location. Each half consisted of a smaller anterior, or head kidney, and a larger posterior, or trunk kidney. The head kidneys appeared as two bilateral oval masses well-demarcated from the posteriorly seated trunk kidney (Fig. 1). The trunk kidneys were intimately related to each other, and each kidney appeared clearly separable into an anterior thick portion and a posterior thin portion intervened by a three-sided ventral lobe (Fig. 1).

Microscopic appearance of the kidney of common carp

The head kidney of common carp was made up of hematopoietic tissue surrounding large blood vessels (Fig. 2A). The hematopoietic cells consisted of nucleated oval-shaped erythrocytes, thrombocytes, granulocytes, monocytes, and lymphocytes (Fig. 2B). The latter exhibited a low cytoplasmic nuclear ratio and revealed different sizes. The trunk kidney was composed of several nephrons and fewer heterotopic thyroid follicles containing acidophilic colloid exudate separated from each other by the interstitial hematopoietic tissue (Fig. 2B). The nephron consisted of the following parts including Bowman's capsule, proximal convoluted tubule, intermediate segment, and distal convoluted tubule (Fig. 2 C,D). The Bowman's capsule appeared as a cup-shaped structure surrounding a tuft of capillaries, the glomerulus. The proximal convoluted tubule revealed a wide lumen and was lined by a simple cuboidal epithelium carrying microvilli with large round nuclei and secretory cytoplasm. The intermediate segment was lined by low columnar ciliated epithelium. The distal convoluted tubule had a narrow lumen with no brush border and course cytoplasmic granules. The interstitial hematopoietic tissue was lodged among different nephron segments and composed mainly of nucleated erythrocytes, thrombocytes, granulocytes, and lymphocytes (Fig. 2D).

Flow cytometry analysis of hematopoietic cells in the kidney of common carp

Flow cytometry analysis of the cellular populations of the kidneys of common carp following H33342 staining revealed the G0/G1 subpopulation to be more enriched in the head kidney compared to the trunk kidney (97.8% vs. 82.8%) (Fig. 3A, B). Conversely, the proportions of S/G2/M and SP cells were less abundant in the head kidney compared to the trunk kidney (0.6% and 0.8% vs. 4.8% and 2.8%, respectively) (Fig. 3A, B). The forward (cell size) and side (cell complexity) scatters of the cells are shown in (Fig. 3C, D).

Flow cytometric study of hematopoietic cells from the head kidney (pronephros) (Fig. 4A) and trunk kidney (mesonephros) (Fig. 4B) of common carp stained with Hoechst 33342. After gating, FSC (forward scatter) vs. SSC (side scatter) there are three distinct populations: erythrocytes, lymphocytes, and thrombocytes (E&L&T) (FSlow, SSlow), which have small cell size and little granularity; monocytes (M) (FShigh, SSlow), which have large cell size and little

granularity; and granulocytes (G) (FSmid, SShigh), which have medium cell size and much granularity.

Flow cytometric examination of hematopoietic cells in the head kidney (pronephros) (Fig. 5A, C) and trunk kidney (mesonephros) (Fig. 5B, D) of common carp to determine the counts of CD34-expressing cells and CD41-expressing cells was shown in Fig. 5. It was obvious that the trunk kidney (mesonephros) had more CD34- and CD41-expressing cells than the head kidney (pronephros), indicating that it has more hematopoietic stem cells.

Immunohistochemical localization of hematopoietic cells in the kidney of common carp

Immunohistochemical analysis of hematopoietic cells in the kidneys of common carp using a specific antibody against CD34 showed the CD34 immunoreactive cells in the head kidney to be dispersed through its parenchyma (Fig. 6A). While in the trunk kidney, the CD34 immunoreactive cells were concentrated close to the basement of renal tubules (Fig. 6B). No CD34 immunoreactive cells were detected in the negative control sections, in which the primary antibody was omitted (Fig. 6C).

Discussion

Many studies have explored the structure, morphocytochemistry, histochemistry, immunohistochemistry, and ultrastructure of the stromal and cellular components of the head kidneys (pronephros) in teleosts [25,32-36] and trunk kidneys (mesonephros) [25,37-40].

The kidneys of common carp have anatomical features that are similar to those found in other teleost species [41,42]. However, as observed by [43-45] , Teleost kidney morphology shows considerable variation. In common carp, the kidney is classified as type II, which is characterized by slender, elongated organs that have a dark reddish-brown color. These kidneys are located on the dorsal side of the swim bladder and ventral to the spinal column, situated within the abdominal cavity. They are divided into two parts: the anterior head kidney (pronephros) and the posterior trunk kidney (mesonephros). The head kidney consists of two oval masses. In contrast, many fish species, such as salmonids, centrarchids, acipenserids, and cichlids, lack this bilateral structure [41,46,47]. The head kidney consists of two awesome oval loads which might be separated from the trunk kidney [48] .The trunk kidney is made up of a robust anterior section and a more slender posterior section, with a three-sided ventral lobe that rests on the swim bladder isthmus. Kondera [23] found comparable traits in carp, but classified the lobes as two tubular structures.

The pronephros of the common carp is histologically composed of hematopoietic tissue, which is supported by a network of reticular cells. This fibrillary structure is present throughout the head kidney in all teleosts and several organs with hematopoietic functions observed in Chondrichthyes [49,50]. Hematopoietic cells are arranged around blood vessels and can be recognized by their unique features: large, round, or oval nucleated red blood cells; small, oval-shaped platelets with a lower nucleus-to-cytoplasm ratio; large granulocytes that have lobed nuclei and reddish cytoplasm; and lymphocytes and monocytes, which come in various sizes. Lymphocytes can be mistaken for bigger cells that contain hemosiderin and melanin, and they frequently form melanomacrophage centers for pigment recycling. Similar results have been observed in other fish species [15,17,51].

The head kidney of the common carp is made up of hematopoietic, lymphoid, and endocrine tissues [52]. Common carp head kidneys, like those of other teleosts, lack renal tubules). The head kidney develops from the pronephros, where the initial tubules expand throughout the early stages of growth but eventually disappear when the mesonephros takes over excretory function [23,53,54].

The trunk kidney, also known as the mesonephros, is made up of renal tubules (nephrons), interstitial hematopoietic tissue, and some scattered heterotopic thyroid follicles, according to Roberts [55], The nephron is the trunk kidney's functional unit, consisting of renal corpuscles and urine tubules. Bowman's capsule, a cup-like shape, consists of two layers of flattened epithelial cells surrounding the glomerulus. Uriniferous tubules are divided into three sections: proximal convoluted, intermediate segment, and distal convoluted. Interstitial hematopoietic tissue is located between the nephrons and consists of large nucleated erythrocytes, microscopic ellipsoidal thrombocytes, granulocytes, and small lymphocytes. Heterotopic thyroid follicles are characterized by follicular epithelium with a thick, homogeneous colloid exudate [45,56,57].

Monoclonal antibodies (mAbs) are frequently used in flow cytometry to purify hematopoietic stem cells (HSCs) in mammals [58]. A comprehensive flow cytometric analysis of hematopoietic cells in the head (pronephros) and trunk (mesonephros) kidneys of common carp (*Cyprinus carpio*) was performed following H33342 staining. The measurement of Hoechst red in relation to Hoechst blue identified three distinct populations linked to the cell cycle: "S/G2/M," "G0/G1," and a side population (SP), which aligns with observations made in mammals [30]. The "S/G2/M" and "G0/G1" populations represented unusual stages of the cell cycle, as shown by their red and blue H33342 fluorescence intensities. The SP population, distinguished by its low H33342 fluorescence, showed the existence of hematopoietic stem cells. In the apex kidney (pronephros), the proportions were 0.6% for "S/G2/M," 97.8% for "G0/G1," and 0.8% for SP. In contrast, the trunk kidney (mesonephros) had

proportions of 4.8%, 82.5%, and few.8%, respectively. These outcomes were consistent with those found in ginbuna carp [4], zebrafish [59], and medaka [60]. The proportions of kidney SP cells in ginbuna carp, zebrafish, and medaka were 0.17%, 0.096, and 0.18%, respectively.

The flow cytometry analysis of Hoechst 33342-stained hematopoietic cells from the common carp's head kidney (pronephros) and trunk kidney (mesonephros) showed three notable cellular groups. These cells were identified largely based on their forward scatter (FSC) and side scatter (SSC) properties, which suggest cellular size and granularity. Erythrocytes, lymphocytes and thrombocytes were smaller and demonstrated minimal granularity (FSlow, SSlow); however, monocytes were larger, exhibiting low granularity (FShigh, SSlow). In contrast, granulocytes displayed a medium size accompanied by high granularity (FSmid, SShigh). This finding is consistent with similar observations made in other teleost species [20,61,62].

CD34 is commonly accepted as a marker for hematopoietic stem cells (HSCs) in animals [6,63,64], Murine hematopoietic stem cells do not express CD34 [30,65]. CD41 is temporarily present in early hematopoietic progenitors in both humans and animals, and it reemerges later in the lineage of platelets and thrombocytes [66]. This study used flow cytometry to look at hematopoietic cells in the head (pronephros) and trunk kidneys (mesonephros) of common carp. The trunk kidney had a considerably higher number of CD34- and CD41-expressing cells, indicating a higher concentration of hematopoietic stem cells. Immunohistochemistry confirmed these observations, revealing CD34-positive cells spread throughout the trunk kidney near the renal tubules, but more dispersed in the head kidney. Similar findings were seen in other teleost species [5,60,67,68].

Conclusion

This study examined hematopoietic stem cells (HSCs) located in the pronephros and mesonephros of common carp. Through flow cytometry and immunohistochemistry, researchers found a greater concentration of HSCs in the trunk kidney, which serves both hematopoietic and renal roles, whereas the head kidney primarily concentrates on blood cell production. These results deepen our understanding of kidney physiology in fish.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of Faculty of Veterinary Medicine at Mansoura University (ethics approval number; Ph.D/73).

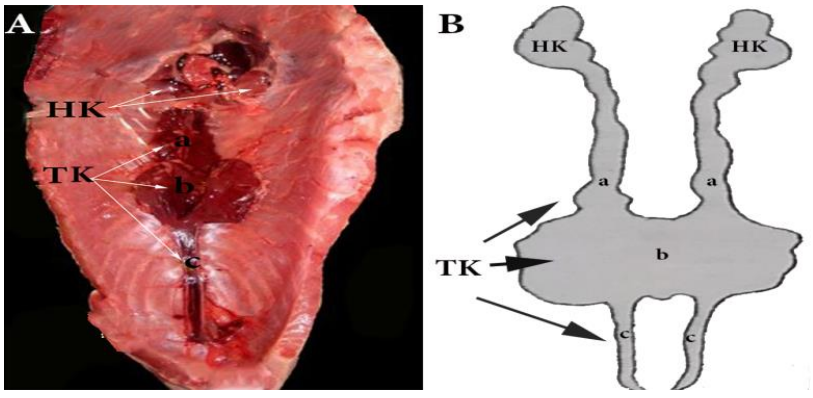

Fig 1. Kidney anatomy and subdivisions in common carp (Cyprinus carpio). A) photograph showing the kidney of the common carp in situ (ventral view). HK, head kidney, TK, trunk kidney having: a-thick cranial part, b-expanded ventral lobe, and c-thin caudal part. B) Diagram for the kidney of the common carp. HK, head kidney, TK, trunk kidney having: a-thick cranial part, b-expanded ventral lobe, and c-thin caudal part.

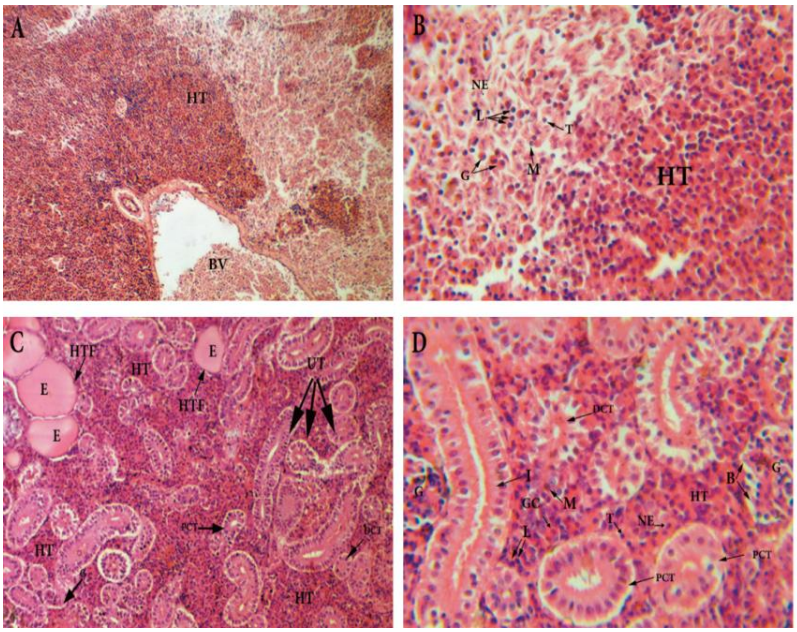

Fig. 2: Light microscopic structure of the kidney in common carp (Cyprinus carpio):

A) Microphotograph of transverse section of head kidney (pronephros) of common carp (Cyprinus carpio) showing HT, hematopoietic tissue, BV, blood vessel X10.

B) Microphotograph of transverse section of head kidney (pronephros) of common carp (Cyprinus carpio) showing HT, hematopoietic tissue, NE, nucleated erythrocytes, T, thrombocytes, G, granulocytes, L, lymphocytes and M, monocytes X40.

C) Microphotograph of transverse section of trunk kidney (mesonephros) of common carp (Cyprinus carpio) showing HT, hematopoietic tissue, HTF, heterotopic thyroid follicle, E, exudate, UT, uriniferous tubule, G, Glomerulus, PCT, proximal convulated tubule, DCT, distal convoluted tubule X10.

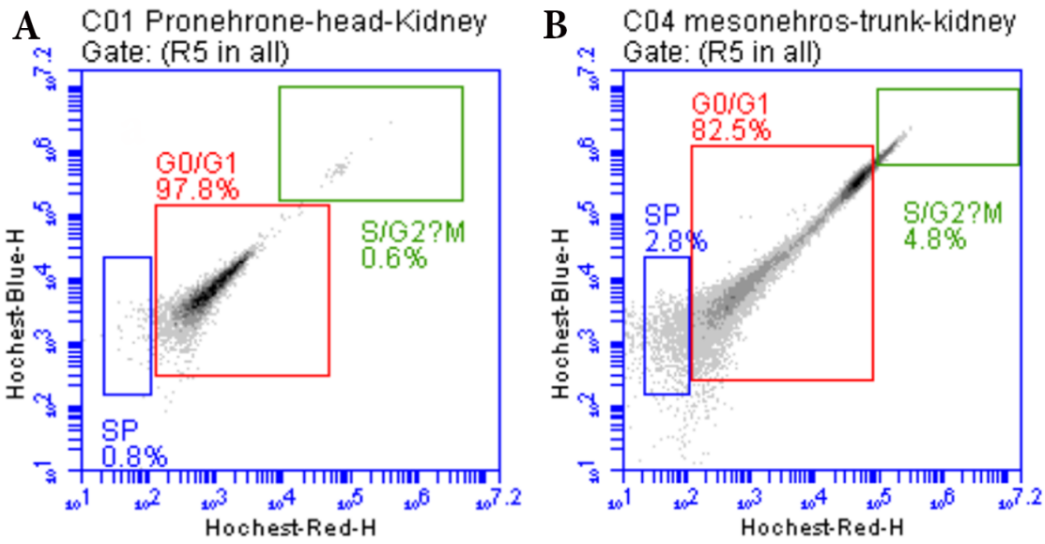

Fig. 3. Flow cytometric analysis of the Hematopoietic cells of common carp (Cyprinus carpio) were stained with Hoechst 33342. showing

A- The hematopoietic cells of Head kidney(pronephros) showing 3 distinct cell populations termed ‘‘S/G2/M’’, ‘‘G0/G1’’, and ‘‘SP’’ their proportions were 0.6 %, 97.8%, and 0.8% respectively.

B- The hematopoietic cells of Trunk kidney (mesonephros) were revealed three distinct cell populations termed ‘‘S/G2/M’’, ‘‘G0/G1’’, and ‘‘SP’’. Which proportions were 4.8 %, 82.5%, and 2.8% respectively.

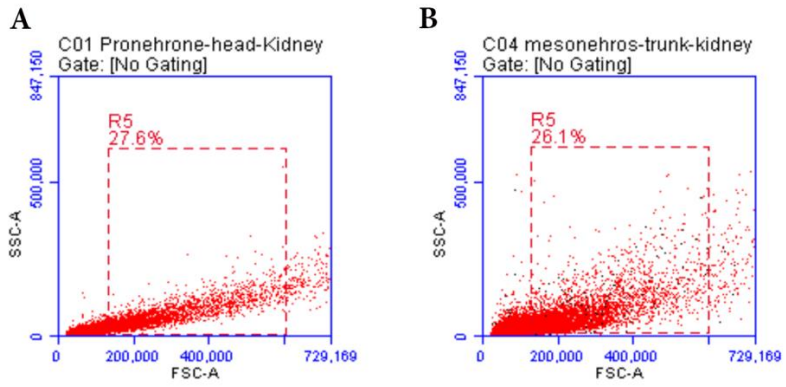

Fig. 4. Flow cytometric analysis of the Hematopoietic cells of Head kidney (pronephros) (A) and Trunk kidney(mesonephros) (B) of common carp (Cyprinus carpio) were stained with Hoechst 33342.
After gating FSC (forward scatter) vs. SSC (side scatter) There are three distinct populations: erythrocytes, lymphocytes, and thrombocytes (E&L&T) (FSlow, SSlow), which have small cell size and little granularity; monocytes (M) (FShigh, SSlow), which have large cell size and little granularity; and granulocytes (G) (FSmid, SShigh), which have medium cell size and much granularity.

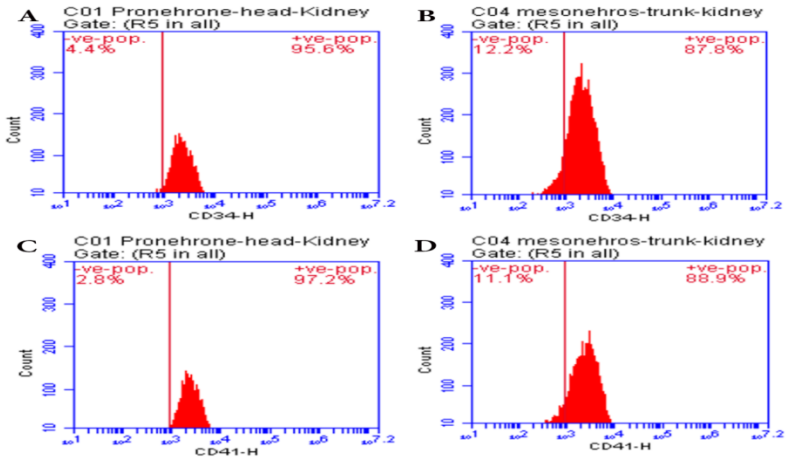

Fig. 5. Flow cytometric analysis of the Hematopoietic cells for determination of CD34-expressing cells and CD41-expressing cell counts in of Head kidney(pronephros) (A, C) and Trunk kidney (mesonephros) (B, D) of common carp (Cyprinus carpio) respectively.

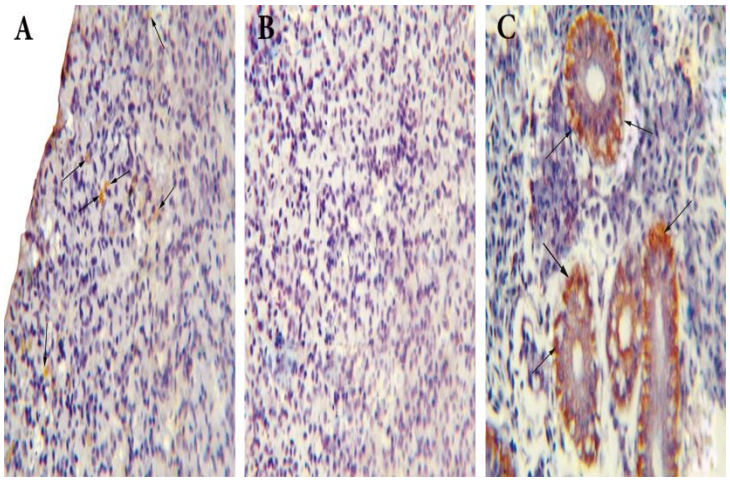

Fig. 6. Immunohistochemistry of CD34 expressing cells in the kidney of common carp (Cyprinus carpio)
A-Head kidney (pronephros): note positive CD34 cells (black arrows)
B-Head kidney (pronephros) control negative (no reaction)
C-Trunk kidney (mesonephros): note positive CD34cells (black arrows) at the basement of the renal tubules.

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التحليل المقارن للخلايا الجذعية المكوِّنة للدم في كليتي الرأس والجذع لسمكة الشبوط الشائع (*Cyprinus carpio*) باستخدام الفحص النسيجي، الكيمياء النسيجية المناعية والتدفق الخلوي

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الملخص

تُعدّ الخلايا الجذعية المكوّنة للدم ذات وظيفة أساسية في إنتاج خلايا الدم لدى الفقاريات. وفي الأسماك العظمية مثل الشبوط (المبروك) الشائع ، تُعد الكليتان لا سيما الكلية الأمامية (الرأسية) والكلية المتوسطة (الجذعية) من الأعضاء الحيوية في عملية تكوّن الدم. هدفت هذه الدراسة إلى فحص توزيع الخلايا الجذعية المكوِّنة للدم عبر هذه المناطق الكلوية المحددة، باستخدام مجموعة متنوعة من التقنيات، شملت الفحص النسيجي، والكيمياء النسيجية المناعية، والتدفق الخلوي.خضعت الأعضاء المكوِّنة للدم المتمثلة في الكلية الرأسية والكلية الجذعية لتشريح دقيق. وقد كشفت الطرق النسيجية المختلفة عن مواضع توزع الخلايا المكوّنة للدم، إلا أن الكيمياء النسيجية المناعية كانت ذات أهمية خاصة في إبراز المناطق التي تُعبّر عن بروتين سطح الخليه34 وعلى الرغم من تنوع الأساليب المستخدمة، فإن هذا النهج الشامل قد أسهم في تعزيز فهمنا لتوزيع الخلايا الجذعية المكوِّنة للدم داخل الكليتين. كما أتاح استخدام صبغة هويشت3342 ، والتي تم تحليلها عبر التدفق الخلوي، دراسة مجموعات خلوية متنوعة ومراحلها المختلفة في دورة الخلية، وهو أمر بالغ الأهمية لفهم هذه العمليات. تتكون كلية الشبوط الشائع من منطقتين رئيسيتين: الكلية الرأسية، والتي تحتوي بشكل أساسي على عدد كبير من الخلايا المكوِّنة للدم المحاطة بالأوعية الدموية، والكلية الجذعية، المعروفة بوجود النفرونات والأنسجة الدموية المرتبطة بها. يحتوي كلا المنطقتين على خلايا دم ذات أنويه، إلا أن عدد الخلايا في طور G0/G1 يبدو أعلى في الكلية الرأسية. وتشغل الخلايا الجذعية المكوِّنة للدم الكلية الجذعية كذلك وهو أمر ذو دلالة نظرًا لأنها تُظهر علامات سطحية للخليه مثل بروتين 34و41 .وتشير الخرائط إلى أن عدد هذه الخلايا الجذعية يكون أوفر في الكلية الجذعية مقارنةً بالكلية الرأسية، ما يثري فهمنا لعملية تكوّن الدم في الأسماك.

الكلمات الدالة: الكلى، الشبوط (المبروك) الشائع، الخلايا الجذعية المكوّنة للدم، التدفق الخلوي.