Comparative histologic anatomy of vertebrate liver

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Abstract

Introduction: The aim of the study was to compare the histological features of the liver of vertebrates and demonstrate the possible histological evolutionary trend within the sub-phylum *vertebrata*. **Materials and Methods:** Liver tissues were dissected from the five vertebrate classes; the Fish (Cat-fish - *Clarias gariepinus*), Amphibian (Rana tigrina), Reptilian (Lizard - *Agama aculeata*), Avian (Domestic bird-*Gallus gallus domesticus*), and Mammalian (Wistar rat- *Rattus norvegicus*) were used for the study. The tissue samples were fixed in 10% formal-saline and processed using standard histological techniques. The liver tissues were studied with a light microscope and photomicrographs were obtained. **Results:** The results showed structural similarities of the liver across the sub-phylum, with noticeable differences in the hepato-sinusoidal arrangements, composition and association of the bile duct and presence of portal triad. The study observed a gradual transition of hepatocytes distributions in singles and clusters to cord-like as observed in Gallus gallus domesticus and *Rattus norvegicus*. The study also revealed incomplete portal triad in *Clarias gariepinus* and *Rana tigrina*. The observed variations were attributed to adaption, feeding mechanism, mode of nutrition and selective specialization, which are important ecological and biological changes involved in evolution. **Conclusion:** This study has established that evolutionary trends occurred within the subphylum vertebrata.

Key words: Hepatocyte, liver, phylogeny, vertebrate

INTRODUCTION

Metabolic activity has been shown to be an important factor that enhances the success of adaptation of vertebrates to their environment. The vertebrate liver is involved in absorption of nutrients from the digestive tract which are subsequently processed and stored. The metabolic functions of the liver include protein synthesis, storage, bile secretion, detoxification and inactivation of harmful substances.[1-5] The basic structural and functional unit of the liver is the acinus, which consist of hepatic lobule and portal triad (also called Glisson's sheath).[3,4] The hepatic lobules are the functional units of the liver.[1,3,4] The sinusoids are capillary networks which are localized in the spaces between hepatic plates.[1,3,4] The liver synthesizes bile which eventually empty into the gall bladder.[1,4]

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A previous study revealed that the liver of various species of fishes demonstrated phylogenic relationships.[6-10] Studies on the phylogenic relationship of the hepato-sinusoidal architecture of the liver of vertebrate subphylum have been reported to be scanty.[8,10-12] The nature and composition of the portal triad have also been described to possess some phylogenic properties.[8,10-12] The aim of this study was to compare the histological features of the liver of vertebrates and demonstrate the possible histological evolutionary trend within the sub-phylum *vertebrata*.

MATERIALS AND METHODS

Ethical approval was obtained from the Research Ethics Committee, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka in strict accordance with the guidelines for the care and use of research animals as approved by W.H.O.[13] The study was carried out in Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka. Twenty (20) animals from the five vertebrate classes, four (4) healthy animals per class were chosen to ensure comparison within and between classes in the study. The classes included *Rattus norvergicus* (mammalian), *Gallus gallus domesticus domesticus* (birds), *Agama aculeata sp* (lizard) and *Rana tigrinas* (frog) and *Clarias gariepinus* (fish). These apparently healthy animals, which had been acclimatized in an animal house for about 2 weeks (certified healthy by the veterinary unit of the college of health sciences, Delta State University), they were administered distilled water for twenty four (24) hours to ensure the gastrointestinal tract was completely evacuated of undigested material. The animals were sacrificed by cervical dislocation, the liver organs dissected and fixed in 10% formal saline for histological processing using standard techniques.[14]

RESULTS

Histological sections of the mammalian liver showed normal hepatic lobules composed of cords of hepatocyte separated by thin fibro vascular connective tissue stroma. The hepatic lobules composed of a polygonal mass of tissue with portal tracts at the periphery and a centrilobular vein in the center. The portal triads were composed of hepatic arterioles, portal venules and bile ductules as shown in Figure 1 and summarized in Table 1.

The sections of bird liver showed hepatocytes disposed in cords and clusters. The hepatocytes possessed centrally placed nuclei. The intervening fibro-vascular connective tissue displayed nucleated red cells with congested hepatic veins and portal triad similar to those of mammals as shown in Figure 2.

Histological section of the reptilian liver showed hepatocytes disposed predominately in singles and clusters, separated by thin fibrovascular connective tissue stroma (sinusoids) in which were nucleated red cells. Individual hepatocytes were observed to have various sized abundant vacuoles within their cytoplasm pushing the nucleus to the periphery with few other cells displaying a centrally placed nuclei and scant cytoplasmic vacoulation as shown in Figure 3. Also seen were portal triads composed of portal veinules, bile ductules and hepatic arterioles [Figure 3].

Sections of the liver of *Rana tigrimas* showed hepatocytes disposed in singles and few clusters by a loose fibrovascular connective tissue stroma as shown in Figure 4. The intervening sinusoids were dilated with plentiful nucleated red cells within their lumen. Individual hepatocytes were found to have abundant eosinophilic and granular cytoplasm and centrally placed nuclei. Also present were portal tributaries hepatic arterioles and bile ductules which were disposed in singles.

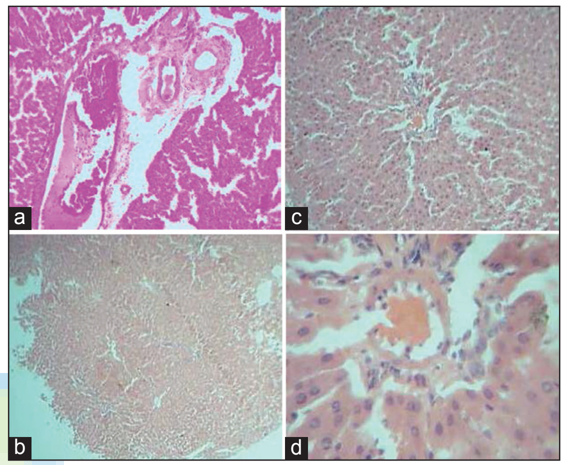

Figure 1: Comparative histology illustrations of the mammalian liver. (a) Section of the Liver of *rattus norvergicus* showing hepatocytes (he), and portal triad (pt) H&E × 100 (b) Section of the Liver of *rattus norvergicus Gallus gallus domesticus domesticus agama aculeata sp rana tigrinas clarias gariepinus* showing hepatocytes (he) disposed in cord and sheet H&E × 40 (c) Section of the Liver of *rattus norvergicus* showing hepatocytes (he) disposed in cord and sheet. A prominent central venules (cv) is present in the center H&E × 100 (d) Section of the liver of *rattus norvergicus* showing hepatocytes (he) disposed in cord and sheet. A prominent central venules (cv) is present in the center H&E × 400

Table 1: A Tabular presentation of histological variations within vertebrates liver

Vertebrate class	Hepatocyte	Portal triad	Nucleated red blood cells	Connective tissue	Bile duct	Biliary duct association
Mammals	Disposed in cords	Present	Absent	Thin fibro vascular connective tissue stroma	Relatively thickened and lined by cuboidal to columnar epithelium	In association with portal triad
Fish	Disposed in sheets, relatively few clusters and singles	Absent	Present	Loose fibrovascular connective tissue stroma	lined by cuboidal to columnar epithelium	Bile ductules in singles and few in association with arterioles or venules
Amphibians	Singles and clusters	Absent	Present	Relatively thin Fatty vacuolated droplets	lined by cuboidal to columnar epithelium	Bile ductules in singles and few in association with arterioles or venules
Reptiles	Singles and clusters	Present	Present	Fatty vacuolated droplets	lined by cuboidal to columnar epithelium	In association with portal triad
Avian	Singles and clusters	Present	Present	Loose fibrovascular connective tissue	lined by cuboidal to columnar epithelium	In association with portal triad

Histological sections of *Clarias gariepinus* liver were observed to consist of hepatocytes disposed in sheets, singles and clusters, each separated by loose fibrous connective tissue stroma on which lies endothelial cells of the liver sinusoids. Individual hepatocytes were composed of centrally placed nuclei with coarse chromatin and abundant granular cytoplasm as shown in Figure 5 Scattered through the liver were branches of the hepatic artery (A), a branch of the portal vein (B) and a branch of the bile ductules in singles. The portal vein tributaries and hepatic arterioles were lined by endothelial cells while cuboidal to columnar epithelia cells lined the bile ductules. The portal triads were indistinct as shown in Figure 5. Nucleated red cells were also seen within the sinusoids.

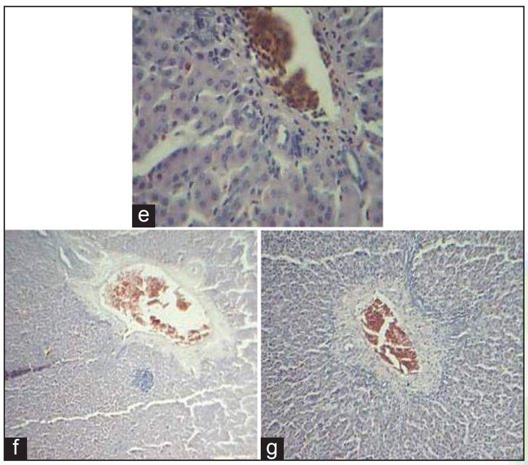

Figure 2: Comparative histology illustrations of the bird. (e) Section of the liver of *Gallus gallus domesticus domesticus* showing a portal triad (pt) composed of a large vein, artery and bile ductile H&E × 400 (f) Section of the liver of *Gallus gallus domesticus domesticus* showing a portal triad (pt) composed of a large vein, artery and bile ductile H&E × 40 (g) Section of the liver of *Gallus gallus domesticus domesticus* indicating central vein H&E × 100

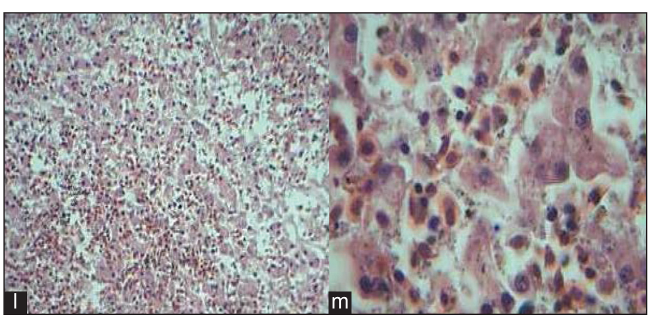

Figure 4: Comparative histology illustrations of the amphibian liver. (l) Section of the liver of *Rana tigrinas* showing hepatocytes disposed in singles and cluster, separated by a loose fibrovascular connective tissue stroma. Nucleated red cells are within the intervening sinusoids H&E × 100 (m) Section of the liver of *Rana tigrinas* showing hepatocytes disposed in singles and cluster, separated by a loose fibrovascular connective tissue stroma. Nucleated red cells are within the intervening sinusoids H&E × 400

DISCUSSION

This study had showed that the histologic architecture of the liver were similar in vertebrate which conforms with those of previous studies.[8,13] however there were marked

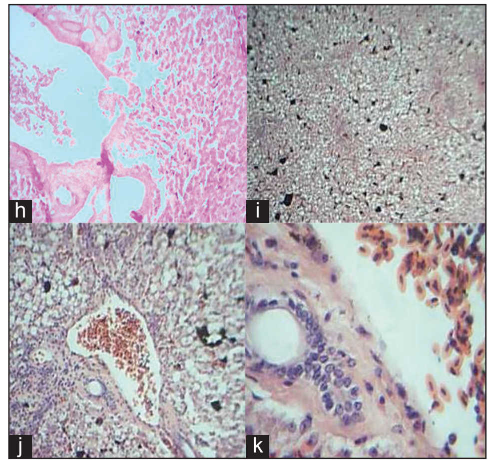

Figure 3: Comparative histology illustrations of the reptile liver. (h) Section of the liver of *Agama aculeata sp* showing hepatocytes (he) in singles and little clusters H&E × 40 (i) Section of the liver of *Agama aculeata sp* showing hepatocytes disposed in singles and little clusters separated by thin fibrovascular stroma. Portal vein, bile ductile and dark staining artefacts are present H&E × 100 (j) Section of the liver of *Agama aculeata sp* showing hepatocytes disposed in singles and little clusters separated by thin fibrovascular strom, dark staining artefacts are present H&E × 40 (k) Section of the liver of *Agama aculeata sp* showing portal vein and bile ductile (bd) with nucleated red blood cells (rb) H&E × 100

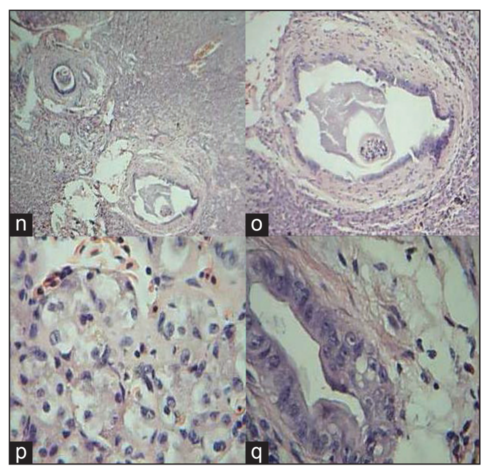

Figure 5: Comparative histology illustrations of the fish liver. (n) Section of the liver of *Clarias gariepinus* showing sheets and clusters of hepatocytes H&E × 40 (o) Section of the liver of *Clarias gariepinus* showing a biliary duct within the liver parenchyma with kuffer cell (kc), H&E × 100 (p) Section of the liver of *Clarias gariepinus* showing hepatocytes disposed in clusters (he). Few nucleated red cells (rc) are seen in the background H&E × 400 (q) Section of the liver of *Clarias gariepinus* showing a biliary duct (bd) with straitified columnar epithelium. Few hepatocytes and nucleated red cell are seen in the periphery (H&E × 400)

differences, which was attributed to species specific metabolic activities, adaptational changes, mode of nutrition and phylogenic organizational perculiarities.

Elias and Bengelsdorf, hypothesized a method of classifying the liver into several groups which served as base line, in conducting this study.[14] Histological analysis of the liver showed that the hepatocytes were disposed in singles to cord like form across the subphylum vertebrata. This distribution of the hepatocytes was attributed to phylogeny as was observed in the index study in which the hepatocytes of *Clarias gariepinus* demonstrated the most primitive distribution of liver cells. This organisms hepatocytes were disposed in singles predominantly, other sections showed hepatocytes in sheets as had been previously documented.[8,10,12]

The distribution of liver cells were however different in *Rana tigrinas* in which the hepatocytes were disposed in singles and clusters similar with those of *Agama aculeata,* except in the cytoarchitecture which revealed peripherally disposed nuclei in *Agama aculeata.* The index study therefore surmised that the nucleus of the liver cells of *Agama aculeata* were displaced to the periphery owing to the presence of various sized abundant vacuole within the cytoplasm. The presence of lipid droplets within the liver cells of *Agama aculeata* could be attributed to an adaptive structural modification to sustain the storage functions of the liver animals in this group as previous studies had earlier indicated that the liver stored several substances including glycogen, lipids, vitamins and iron.[1,3,7]

The observed variations within the vertebrate subphylum could be explained by the metabolic activities which have developed by adaptation. Previous studies showed that the fat droplet seen in reptiles could be converted (gluconeogenesis) and used as energy in unfavourable weather or dormancy.[3,8,10] These changes were most likely established as vertebrates transcended the aquatic to terrestrial habitat. Histologic analysis of the liver of *Gallus gallus domesticus* showed hepatocytes disposed in clusters and cords a transition to the predominantly cord like form of hepatocytes seen in *Rattus norvergicus* (Mammals) and clusters demonstrated in higher mammals. The liver cells of *Rattus norvergicus* however revealed polyhedral hepatocytes with eosinophilic cytoplasm, a result of plentiful mitochondria and extensive smooth endoplasmic reticulum.[12,15]

The hepato-sinusoidal arrangement have been largely attributed to evolutionary trends of vertebrates, for as metabolic functions (such as synthesis of plasma proteins, fibrinogen and prothrombin and the regulation of blood glucose and lipids) increased, the vertebrate liver evolved more complex cordlike patterns seen in mammalian species to entertain the metabolic requirements of the body.[1,10] The liver has been reported to function in the deamination of amino acid into urea which enters the blood stream to be excreted via the kidneys.[16-18] Similarly, the macrophage cells (of kupffer) lining the sinusoids of the liver have been reported play a key role in the removal of damaged erythrocytes from the circulatory system.[16,18]

In *Clarias gariepinus,* the biliary ductules were found in isolation, other sections showed bile ducts in association with the venules. The bile ductules displayed a relatively thickened wall lined by cuboidal to columnar epithelium, which was attributed to the mode of nutrition.[6] The index study demonstrated the presence of bile ductules with thick walls which could be a result of the composition of bile salts in cat fish unlike other vertebrate. The bile ducts in *Agama aculeata* were associated with portal triads disposed in acinar, which was similar to those of higher vertebrate classes. Bile ductules were found to be lined by cuboidal or columnar epithelium and displayed distinct connective tissue sheets as observed in lower vertebrates. In *Rattus norvergicus* the bile ductules were found in association with portal triads and anucleated red cells. The presence of anucleated red cells in rats (mammal), unlike was demonstrated in other classes of vertebrates, further confirmed the advancement of the mammalian class.

In conclusion, this descriptive analysis has demonstrated extensive variations and some similarities in the five classes of vertebrate liver histologic architecture. The common hepatic features, albeit few, has further established the existence of strong phylogenic characteristics existing within the vertebrate class.

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