Morphological and histomorphometrical study of the Sacculus rotundus at different postnatal ages in indigenous rabbit

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Summary

Current investigation was carried out to explore structural developmental changes of the acculus rotundus in indigenous rabbits at different postnatal ages. Morphological, histological and morphometrical approaches were employed on 24 rabbits at ages one, ten, fifteen and forty days. The findings indicated that in one day kits, the Sacculus rotundus cannot be recognized with bare eye but in the next advanced ages it appeared as expanded pear-like structure situated at the end of ileum. It opened into the 2nd gyrus at the base of cecum by saccorotundocecal orifice. Because it extends for 1-2 millimeters inside the lumen of the cecum, an annular ridge formed encircles the site of this opening. Length and width of SR increased significantly by age progression. The main four layers of the alimentary canal were presented in the wall structure of Sacculus rotundus but with apparent variations in its structural constituents and dimensions. Two types of epithelia were recognized in its mucosa, villus epithelium and follicular associated epithelium. The lamina propria was very thick and full of well-developed nodular and diffuse lymphoid tissue alongside the whole circumference of its wall even in the newly born kits. In conclusion, the SR might be considered as primary lymphoid organ in rabbit.

Keywords: Morphological, Histomorphometrical, Sacculus rotundus, Postnatal ages, Rabbit.

Introduction

Rabbits are usually reared for their meat, fur and for biomedical purposes (1). Rabbits are considered very specific animal models because they have complicated digestive system and they are hind gut fermenters (2). They daily perform cacography, which is the re-ingestion of mucous coated night feces, as a way of recycling cecotrophs that are rich in B vitamins and proteins (3). Actually many researchers postulated that rabbit is a very suitable lab animal for immunological approaches because of its excellent response toward different antigens (4).

Rabbit contains well developed gut associated lymphatic tissue (GALT) compared to other mammalian species (5). These tissues in rabbit are commonly considered to constitute Payer's patches, Sacculus rotundus, cecal patches and appendix (6). In rabbits the distal end of the ileum showed a spherical thick-walled enlargement known as SR beside that the presence of abundant GALT (7). The earlier structure marks the junction between the ileum, cecum, and colon. The SR is often named as "cecal tonsil" because of its lymphoid tissue composition. Few of previous studies regarding the lymphoid structures in the rabbit were focused on such structure (8). In fact this organ is unique structure to the rabbits (1), while in other animals such as ruminants, pigs and dogs, the GALT existed as Payer's patches only (6 and 9).

Immunological (10), pharmacological (11) and pathological (12) studies were carried out previously on the SR, therefore, the exact histomorphological knowledge on the SR is necessary for further studies and for proper diagnosis of its pathologic disorders (1). There is paucity of works concentrated on the rabbit SR and its lymphoid structure as well as absence of postnatal developmental researches on it up to date. Accordingly current study aimed to elucidate the morphological and histomorphometrical developmental changes in the structure of this organ.

Materials and Methods

Twenty pregnant does were kept and maintained under well laboratory conditions and allowed free access of food (standard basal diet and greenish food) and tap water

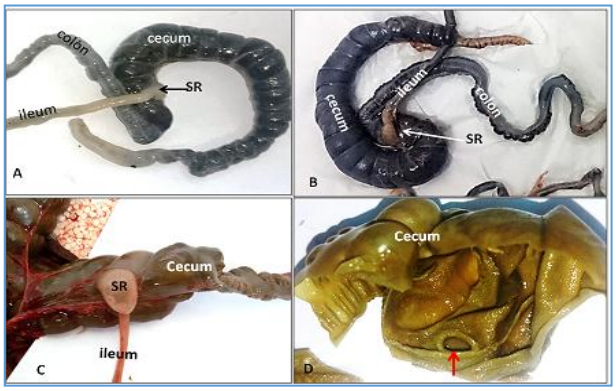
ad libitum. They were purchased from animal house of the College of Veterinary Medicine/ University of Baghdad. From these does, 24 offspring at different postnatal ages (regardless to sex) were collected and set equally into four groups. The newly born puppies of one day age were set in the first group (G1). The second group involved ten days of age which was set as suckling (G2). The third group of fifteen days of age of a period of suckling and start feeding on food which was set as pre-weaned rabbits (G3). Whereas, the fourth group of forty days of age was set as post-weaned animals fed on solid basal diet and greenish (G4). Weaning was set on day thirty of age.

Animals were euthanized by intra-cardiac injection of over dose of sodium pentobarbital (100 mg/ kg) (13). Abdominal cavity was opened using a surgical scissor, intestine and stomach were viewed and dissected away from the abdomen. The work was permitted by ethic committee under the order no. 2036 on 17/6/2015 at Veterinary Medicine Collage/ Baghdad University. The length and width of SR were measured by using a digital vernier caliper (GI power Co., Ltd./ China). Evacuation of the intestinal content conducted by washing with cold normal saline and then directly immersed in 10% neutral buffered formalin for 48 hrs. The representative specimens of one cm were cut from mid part of SR, subsequently specimens were processed by routine histological method to obtain sections of 6 µm thickness. Stains such as Harris's hematoxylin and eosin and Mallory trichrome were used to stain the prepared sections from all studied ages for general histological purposes (14).

Histomorphometrical approaches: Villi epithelial and follicular associated epithelium (FAE) heights, thicknesses of mucosa, lamina propria, tunica muscularis and the diameters of lymphoid follicles were measured using the color USB 2.0 digital camera (Scope Image 9.0) which is provided with image processing software and connected to light microscope. Computer package (Sigma plot V12.0/ SYSTAT software) was used to conduct the histomorphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed using one way analysis of variance (ANOVA) with significant level set on $P<0.05$ and the differences among the groups were determined by Duncan's multiple range test (15).

Results and Discussion

The gross examination revealed that the distal end of the ileum in rabbit is dilated to form sac-like structure called sacculus rotundus which opens directly in to the cecum. In one day aged kits, the ileum seemed to be continuous with the SR without any visible mark between them, whereas in the suckling and pre-weaned rabbits (aged 10 and 15 days, respectively) there was visible constricted groove remarks the connection between ileum and SR externally. In 40 days aged rabbit (post-weaned), the end of ileum looks like a tube penetrating the wall of SR which extended for about 1 mm into its lumen. The visible SR appeared in rabbits as rounded or pear-like structure and opened into the 2nd gyrus at the base of cecum by saccorotundocecal orifice which extends for 1-2 millimeters to the lumen of cecum to form annular ridge encircling the site of opening. In another aspect, one cecal fold comes to attach with this ridge (Fig.1).



Figure, 1: Hind gut of rabbit showing the development of SR at 10 days (A), 15 days (B) and 40 days (C). D: represent the opening of SR within cecum (red arrow).

In one day aged kits, it was difficult to recognize the SR from the ileum but the wall of its site appeared harder in texture than that of ileum by palpation. In the suckling rabbits, the SR appeared as small, light colored dilatation at the end of ileum and measured 1.2±0.05 cm length and 0.6±0.01cm width (Table, 1). In the later postnatal studied ages, the SR increased in its size to reach about

1.75±0.07 X 0.9±0.04 cm and 2.6±0.02 X 1.7±0.06 cm in the rabbits aged 15 days and 40 days, respectively (Table, 1). In addition to that it became visible and can be recognized easily through its white to pinkish color in contrast to the greenish color of ileum and cecum (Fig. 1).

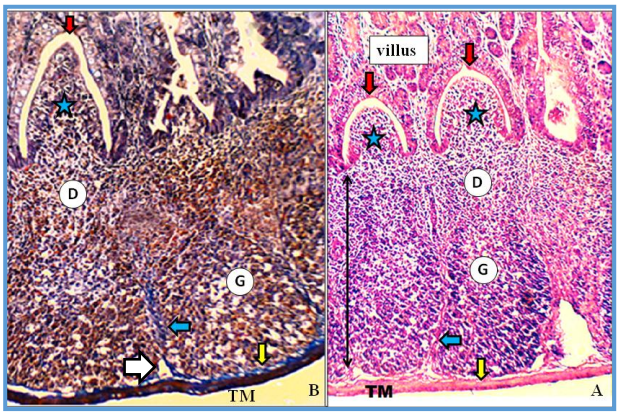
Table, 1: Gross measurements of SR (cm) in rabbits at different post natal ages. ±SE.

Ages parameters	P0	P10	P15	P40
SR length	-	1.2±0.05 a	1.75±0.07 b	2.6±0.02 c
SR width	-	0.6±0.01 a	0.9±0.04 b	1.7±0.06 c

Note: Different letters in rows means there is a significant difference (P <0.05). SE: Standard error.

The characteristic feature of the digestive system of rabbit out of which is the presence of sac-like structure at the end of ileum which they called it sacculus rotundus. It is located at the site which connects ileum with the cecum (3 and 5). Current findings indicated that the SR is opened into the 2nd gyrus of the base of cecum by an opening which extends for about 1-2 mm into the lumen of cecum to form papilla encircled by annular ridge. This finding comes parallel with those observed by (3) in angora rabbit and (5) in Egyptian rabbit, but other researchers such as (7) did not referred to such papilla in their studies on New Zealand rabbit. However, dissimilarly (16) studied previously rabbit cecum and recorded that the SR is opened into the 1st gyrus of the base of cecum with opening they named ileocecal orifice. Currently, the annular ridge did not exist in newly born kits and appeared very clear in the advanced ages. On the other hand, it was found that the opening was bordered by one of the cecal folds which come in contact with the annular ring of it. This finding was in inconsistence with those of (1) in angora rabbit and (17) in New Zealand rabbit and (16) in other domesticated rabbits as they recorded that the opening of SR was bordered by two lateral cecal folds.

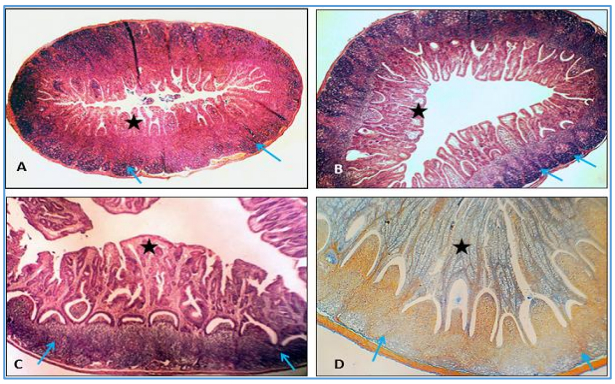
Microscopic examination revealed that the wall of SR had the same known four intestinal tunicae but with distinct variations in their morphology including the irregular shape of villi, obvious increase in thickness of lamina propria with its interesting content of lymphoid tissue (Fig. 2). Beside that very thin submucosa and muscular tunicae with characteristic ill-developed Auerbach's nerve plexus.



Figure, 2: (A) Wall of SR at 15 days of age showed typically 4 tunicae. Not the crescent hallows above the dome-shaped (blue stars), thick lamina propria (double heads arrow) contains diffuse (D) and follicular lymphatic tissue with germinal center (G), tunica muscularis (TM) and thin submucosa (yellow arrow) from which extend the septa (blue arrows) and blood vessel (white arrow). A: H and E, X100, B: Mallory Trichrome, X100.

The villi were raised from the tunica mucosa and extended toward the lumen which covered by simple columnar epithelium with the existence of large number of goblet cells and few enteroeocrine cells. The epithelium enveloped dome shaped lamina propria which covered by crescent hallows. The height of lining epithelium of villi was not changed significantly through postnatal developmental studied ages, whereas the height of FAE which covered the lymphoid follicles was increased gradually at the different postnatal ages up to 15.66±0.7 µm, 18.1±0.41 µm, 18.2±0.23 µm, 27.6±0.9 µm, at the ages of 1, 10, 15 and 40 days, respectively (Table, 2). The villi of SR were characterized by their narrow bases and wide apices. They increased in height during postnatal developing ages and became branched or anastomosed with each other in older studied ages to form irregular or tree-like view (Fig. 3).

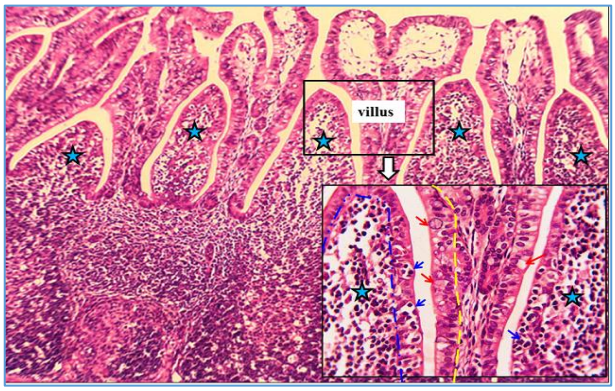
Lamina propria appeared very thick layer and measured 381.45±17.4, 494.03±7.1, 497.16±6.2 and 591.16±14.3 µm in ages of 1, 10, 15 and 40 days, respectively (Table, 2). It showed very interesting lymphoid follicles in addition to diffuse lymphoid tissue distributed along the entire circumference of the wall of SR (Fig. 2).



Figure, 3: Microphotograph through the wall of SR at different postnatal ages: A (1days), B (10 days), C (15days) and D (40 days). Note the shape of villi (black stars) and development of lymphoid follicles (blue arrows). X40, (A, B, C) H and E, (D) Mallory Trichrome stain.

The lymphoid follicles appeared well developed in SR of rabbit and they could be recognized even in the newly born kits but they were showed ill-defined germinal centers. In the subsequent studied ages, the follicles developed and possess well-defined germinal centers and showed significant increase in their diameters which measured 174.2±13.9 µm, 286.4±14.3, 288.5±17.8 µm and 350.1±16.2 µm in the postnatal ages of 1, 10, 15 and 40 days, respectively, the difference between 10 days aged rabbits and 15 days was non-significant (Table, 2). Obvious connective tissue trabeculae were found originating from the tunica submucosa and extended to separate the lymphoid follicles from each other (Fig. 2). The lamina propria bulged into the lumen to form dome-shaped structures located among the bases of the villi and covered with (FAE) which appeared different in structure to the neighboring villus epithelium. The FAE showed low columnar palely stained cells with pale nuclei that were microfold (M) cells. These cells were detected at all regions of FAE even at the mouth of each crypt of Lieberkühn. Characteristically, this epithelium was free of goblet cells and in some locations possesses solitary goblet cells. In another aspect, this epithelium was invaded with large number of intraepithelial lymphocytes and in some locations appeared as clusters of lymphocytes especially nearby the M cells which form pockets to enfold such clusters (Fig.4). The characteristic feature of these dome-structures is the crescent shape hallows that separate its apices from the bases of the villi (Fig.2). In newly born kits, the tips of these dome structures appeared rounded in shape and became pointed in the ages of 10 and 15 days, whereas they return to be rounded in the rabbits of 40 days. Muscularis mucosa was unapparent in one day newly born kits and it was difficult to recognize the smooth muscle fibers at the deep part of lamina propria.

Tunica submucosa was very thin layer (about 6-10 µm thick) (Table, 2) of connective tissue fibers with few number of blood vessels. Tunica muscularis appeared thin layer consist of one layer circular smooth muscle fibers in newly born kits but in the age of 40 days showed thin inner circular and outer longitudinal layers of smooth muscle fibers. Missonier's nerve plexus could not be recognized in submucosa of all studied ages, whereas, the Auerbach's plexus appeared as ill-developed structures in the connective tissue between the two layers of tunica muscularis in the rabbits aged 40 days and could not be found in other ages.



Figure, 4: Wall of SR of 10 days suckling rabbits showing FAE (blue line) of dome -shape structure (blue stars) with M cells (blue arrows) and the opposite villus epithelium (Yellow line) with goblet cells (red arrows). H&E, X100, X400 (magnified rectangle).

Compared to other mammalian species, rabbits have very well-developed gut associated lymphoid tissue GALT which consist of payer's patches of ileum, SR, cecal patches and the appendix (6 and 18). Characteristically, it was found that the SR in local rabbits different from the rest of the small intestine by having well developed nodular and diffuse lymphoid tissues situated in thick lamina propria. Thick lamina propria was detected alongside entire circumference of its wall, even in the newly born kits and such findings establish the hypothesis that the SR in local rabbits is primary lymphoid organ.

Current records were in agreement with postulations (5, 18 and 19) when they were referred to the novelty of SR as harderian gland. Similarly more than one decade before, (20) described the SR and appendix in young rabbit as similar as the avian bursa of fabricius in the development of B lymphocytes and they also considered them as primary lymphoid organs. On the other aspect, the present results were different from those of (17 and 21) because they described the SR as a muscular organ present at the end of ileum of rabbit. The difference was because the muscular layer of the wall of SR found very thin in local rabbits. In the newly born kits, the lymphoid follicles were presented as small follicles with ill-defined germinal center, but with the advancement of age, they increased in size and separated from each other by trabecular connective tissue and acquired a definitive germinal centers and cortical zones, as well as, the dome-shape structures developed over each follicle and the area between neighboring follicles were filled with diffuse lymphoid tissue. These results were in agreement with (5) who refers to the presence of numerous lymphoid follicles, domes and interfollicular areas at all stubdied ages of rabbit ileocecal patches. Current developmental changes may reflect the immunological role of this organ with the advanced ages and changes in the animal diet from milk to greenish or herbs with cellulose constituents.

Table, 2: Micromorphometric measurements of different parameters of SR/ µm at different postnatal ages in rabbit. ±SE.

Age / Parameters	P0	P10	P15	P40
Epithelium height	24.88±0.08 a	24.75±0.45 b	25.21±0.75 b	25.57±0.4 b
Villi height	288.66±13.0 a	593.23±14.0 b	596.52±18.7 b	1164.2±25.7 c
FAE height	15.66±0.7 a	18.1±0.41 b	18.2±0.23 b	27.6±0.9 SE c
Lamina propria thickness	381.45±17.4 a	494.03±7.1 b	497.16±6.2 b	591.16±14.3 c
Mucosa thickness	670.11±24.9 a	1089.83±25.1 b	1093.68±33.2 b	1755.36±37.7 c
Lymphoid follicle diameter	174.2±13.9 a	286.4±14.3 b	288.5±17.8 b	350.1±16.2 c
Tunica muscularis thickness	18.6±0.9 a	25.12±0.7 b	26.02±0.7 b	44.19±1.7 c
Wall thickness	704.11±2.2 a	1126.65±4.6 b	1129.68±2.1 b	1810.36±3.6 c

Note: Similar letters in rows means absence of significant difference (P<0.05) among groups, whereas different letters in rows means significant difference (P<0.05).

Present study reported two types of epithelia presented in the mucosa of SR, the 1st one was the columnar epithelium with goblet cells that cover the short villi in the newly born kits which increased with the age advancement and became tall anastomosing villi with narrow bases and wide apices. The 2nd epithelium is the FAE that cover the dome-shapes over the lymphoid follicles which consist of short columnar cells, M cells and contain large number of intraepithelial lymphocytes and was devoid of goblet cells. This result was in agreement with that of (22). The epithelium that covers the villi is specialized for absorption and secretion as well as protection whereas the FAE might provide both protective barriers over the lymphoid follicles and sampling of antigens from the gut lumen and deliver them to underlying immune cells in the lymphoid follicles.

In conclusion, current histomorphological findings indicated that the SR act as primary lymphatic organ even in the newly born kits and properly play a key role in immunological responses toward different antigenic stimuli.

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دراسة شكلية ونسجية قياسية للكيسة المستديرة بأعمار مختلفة بعد الولادة في الأرنب المحلية.

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الخلاصة

إن هدف هذا البحث لاستكشاف التغيرات الحاصلة قي تركيب الكيسة المستديرة في أعمار مختلفة للمرحلة التطورية بعد الولادة في الارانب المحلية. أجريت دراسة شكلية ونسجية وقياسية على 24 ارنباً باعمار مختلفة بعد الولادة (1 و10 و15 و40 يوم) . أظهرت النتائج عدم إمكانية تمييز الكيسة المستديرة بالعين المجردة في اليوم الأول من العمر بعد الولادة قي حين ظهرت في الأعمار اللاحقة كتركيب متوسع كمثري الشكل يقع عند نهاية اللفائفي ويفتح في التلفيف الثاني من قاعدة الأعور بفتحة الكيسة المستديرة الأعورية. وبسبب امتدادها لمسافة 1-2 ملم داخل تجويف الأعور، فقد لوحظ تكون حيد دائري يحيط بالفتحة. لوحظت زيادة معنوية في طول وعرض الكيسة مع تقدم العمر. تكون جدار الكيسة المستديرة من الغلالات الأربعة الرئيسة المكونة لجدار

القناة الهضمية لكن لوحظ وجود اختلافات واضحة في تراكيب هذه الغلالات وأبعادها. وقد مُيز نوعان من الظهارة في الغلالة المخاطية، حيث كانت الصفيحة المخصوصة سميكة جدا وممتلئة بنسيج لمفي عقيدي ومنتشر ممتدة على محيط جدار الكيسة المستديرة وقد لوحظ ذلك حتى في عمر يوم واحد. يمكن أن نستنتج أن الكيسة المستديرة عضو لمفي أساسي في الأرانب المحلية.

الكلمات المفتاحية: شكلية، نسجية قياسية، الكيسة المستديرة، اعمار مختلفة بعد الولادة، الأرنب.