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Postnatal developmental studies on the parathyroid gland of male Guinea pig with relation to bone formation

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ABSTRACT

Blood calcium level can be controlled by the action of parathyroid gland on the bone via parathyroid hormone that responsible for bone mineralization. The study aimed to demonstrate the developmental histological structure of parathyroid gland and bone from one day after birth to twenty-four month. Forty-five male Guinea pigs, immature and mature were used for this study. Samples were taken from parathyroid gland and bone and processed for histological examination. Parathyroid gland formed of fibrous capsule and fine septa extending to cellular parenchyma which contained both chief and oxyphil cells. From age of 1 day to 17 month, only chief cells with no differentiation were present in parathyroid gland. Chief cells cytoplasm might be granular, vacuolated, or empty according to secretory activity of cells. At 18 month, oxyphil cells started to appear however, chief cells were differentiated into dark and light cells at 24 months. Compact bone was characterized by regular bone lamellae around vascular Haversian vessels that penetrated the bone instead of Haversian canals in Guinea pig. At early life, bone was not completely ossified forming from hyaline cartilage and spongy bone. Vertebrae were completely ossified at 4 month of age and consisted of spongy bone. At 2 weeks and 1 month, femur and ribs were completely ossified consisting of compact bone.

1. INTRODUCTION

The Guinea pig (*Cavia porcellus*) was one of family Caviidae of rodents (Burnie, 2008). In different countries of Africa, they used as a food (Meredith and Redrobe, 2010). Also, they had a great role in experimental studies as they used as a laboratory animal (Gad, 2013). Most of mammals had the same histological structure of Guinea pig parathyroid gland (Nagpal *et al.,* 1989; Ramayya *et al.*, 2012). Therefore, investigating the histological structure of parathyroid gland of Guinea pig will be a good reference for other animal species.

Parathyroid hormone (PTH) and bone were responsible for calcium homostasis (Carafoli, 2003 and Ramasamy, 2006). Sixty-five percent of bone consisted of calcium and phosphorus that responsible for bone hardness (Ciocca *et al.,* 2015; Dermience *et al.,* 2015).

In most of mammals, chief and oxyphil cells were the main component of parathyroid gland. Chief cells were the main parenchymal cells, arranged in form of cords and were surrounded by blood capillaries and sinusoids (Mini and Manju, 2017). They were two types of chief cells; dark, active and light, inactive chief cells (Enemali *et al.,* 2017). Oxyphil cells were large polygonal cells with strongly acidophilic cytoplasm (Mini and Manju, 2017). In comparison with chief cells, oxyphil cells were larger in size and fewer in number than chief cells. They appeared at puberty and increase in number with age (Gerard, 2006; Enemali *et al.,* 2017).

Histologically, compact, and spongy bones were the main types of bone, consisted of four types of cells are osteogenic cells, osteoblast, osteocyte, and osteoclast cells. Haversian canals were the characteristic feature of compact bone with regular bone lamellae, while spongy bone was characterized by bone marrow spaces with irregular bone lamellae in between (Bashandy and Elharoun, 2014). Haversian canals were absent in some species as rat and Guinea pig and replaced by several vessels called Haversian vessels that penetrated the bone (Bagi *et al.,* 1997; Bashandy and Elharoun, 2014).

This study aimed to demonstrate the different developmental histological structure of parathyroid gland and bone.

2. MATERIAL AND METHODS

The present study was carried out on 45 male healthy Guinea pigs in the Department of Histology, Faculty of Veterinary Medicine, Benha University, Egypt.

2.1. Animals

According to Harkness *et al.* (2010), male Guinea pigs reached sexual maturity between 8 and 10 weeks. Therefore, ages of immature and mature animals were considered as presented in table (1).

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2.2. Sampling

Guinea pigs of different ages were obtained from experimental animal house at the Zoo, Giza Governorate, Egypt. The animals were examined before processing to be free from any disease. The specimens were taken from parathyroid gland and bone. The bone specimens were taken from femur, ribs, and vertebrae of each animal. Guinea pigs were euthanized then opening the animal to obtain parathyroid gland and bone. Parathyroid gland was obtained from the neck region from the middle third of tracheal rings with thyroid gland. However, bone was obtained via opening the animal from the thorax till vent.

Table 1 Animals age, stage of growth and specimens

Age	No. of specimens	Stage
1 day	4	
2 weeks	6	Immature stage
1 month	3	
2 month	6	
4 month	9	
8 month	3	
10 month	4	Mature stage
12 month	4	
18 month	3	
24 month	3	

2.3. Light Microscopy

The parathyroid specimens were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax.

The bone specimens were fixed in 10% neutral buffered formalin for 24-48 hours, then subjected to decalcification with decalcifying agent, 5% Hydrochloric acid. Decalcification occurred at 4°C with continuous shaking and continuous renewal of decalcifying agent daily till the bone became soft. Decalcification time was recorded. After that, the bone specimens were dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax.

Sections of about 5µm thick were obtained from all specimens and stained, according to Bancroft and Gamble (2002). The following stains were used:

- Harris's hematoxylin and eosin stain for general histological structure.
- Periodic acid Schiff technique (PAS) for demonstration of neutral and some acidic mucopolysaccharides.
- Masson's trichrome stain for identification of collagen fibers.
- Picro-thionin stain for identification of bone lacunae and canaliculi.
- Von-kossa stain for demonstration of calcium deposits.

3. RESULTS

3.1. Parathyroid gland

From 1st day till 13rdday, Guinea pig parathyroid gland was surrounded by connective tissue capsule. It was overcrowded at the center than the periphery (Fig.1(A)). There were irregularly arranged oval and round cells with granular or vacuolated empty cytoplasm at the periphery. Cells at the center of the gland were numerous round cells contain large dark nucleus and cytoplasm (Fig.1(B)).

From 14th day to 29th day, cells of Guinea pig parathyroid gland appeared in form of follicles toward the periphery, while they were irregularly arranged at the center (Fig.1(C)). There were highly dividing cells with spherical vesicular nucleus that contained finely dispersed chromatin at the periphery of gland. Cells forming the follicles were round in shape with basophilic cytoplasm either granular or vacuolated and large dark nucleus (Fig.1(D)).

At 1-month till 3-month, parathyroid gland was surrounded by capsule of connective tissue consisted of collagen fibers (Fig.1(E)). Parathyroid gland contained large cells with large dark and euochromatic nuclei. The cytoplasm may be faint granular or empty cytoplasm. Also, there were several fibroblasts between parathyroid cells (Fig.1(F)). Parathyroid cells were PAS positive cells (Fig.2(A)).

The parathyroid gland of 4 till 17-month, parathyroid gland became more crowded with cells (Fig.2(B)). Lymphocytes were present in the parenchyma (Fig.2(C)). Parathyroid cells were polyhedral in shape with spherical dark or light nuclei and granular basophilic cytoplasm (Fig.2(D)). Some cells contained vacuolated cytoplasm (Fig.2(E)).

At 18 month, oxyphil cells began to appear as large polyhedral cells with acidophilic cytoplasm between chief cells (Fig. 2(F)).

At 24 months, parathyroid gland was completely embedded within thyroid gland (Fig.3(A)). The periphery of the gland contained round cells with granular cytoplasm and euchromatic nuclei contain fine chromatin. Also, large round cells with large spherical nucleus and either vacuolated or empty cytoplasm were present (Fig.3(B)). Parathyroid gland was characterized by presence of both types of cells, chief and oxyphil cells. Chief cells were divided into light and dark cells. Light cells were spherical cells with spherical nucleus and pale cytoplasm. Dark cells had spherical shape with large central nucleus and dark granular cytoplasm (Fig.4(C)). Oxyphil cells increased in number than those of 18 months. They were fewer and larger than chief cells. They became arranged either singly or in small groups between chief cells. They possessed acidophilic cytoplasm and oval or round small heterochromatic nuclei (Fig.4(D)).

3.2. Bone

From 1st day till 3rd month, bone was not completely ossified form from both hyaline cartilage and spongy bone. Cartilage differentiated into bone through several stages as resting zone, growth zone, hypertrophic zone, calcification zone and ossification zone. Resting zone was formed from chondrocytes inside lacunae, Growth zone had dividing chondrocytes arranged in form of columns, Hypertrophic zone contained enlarged chondrocytes, Calcification zone consisted of empty chondrocytes and ossification zone had osteoblast, osteocytes and bone marrow spaces (Fig.5(A)).

Vertebrae at 1st day to 3rd month were not completely ossified composing of hyaline cartilage and spongy bone. Spongy bone was characterized by several bone marrow spaces with irregular bone lamellae in between (Fig.5(B)). They were completely ossified at 4 month of age that consisted of spongy bone only (Fig.5(C)).

From 1st day till 13rd day, the femur consisted of hyaline cartilage and spongy bone that contained several bone marrow cavities and irregular bone lamellae (Fig.5(D)). Bone lamellae composed of calcified collagen fibers and osteocytes inside lacunae (Fig.5(E)). Calcified collagen fibers were due to deposition of calcium salts in the boney tissue (Fig.5(F)). Osteocytes connected together through lacunae and canaliculi (Fig.6(A)). Beginning from 2 weeks, the femur was completely ossified consisting of compact bone only which characterized by Haversian vessels with regular bone lamellae (Fig.6(B)).

From 1st day till 29-day, the rib was formed from hyaline cartilage and spongy bone that contained several bone marrow cavities and irregular bone lamellae (Fig.6(C)). At 1 month, rib was completely ossified consisted of compact bone only and medullary cavity at the center of bone (Fig.6(D)).

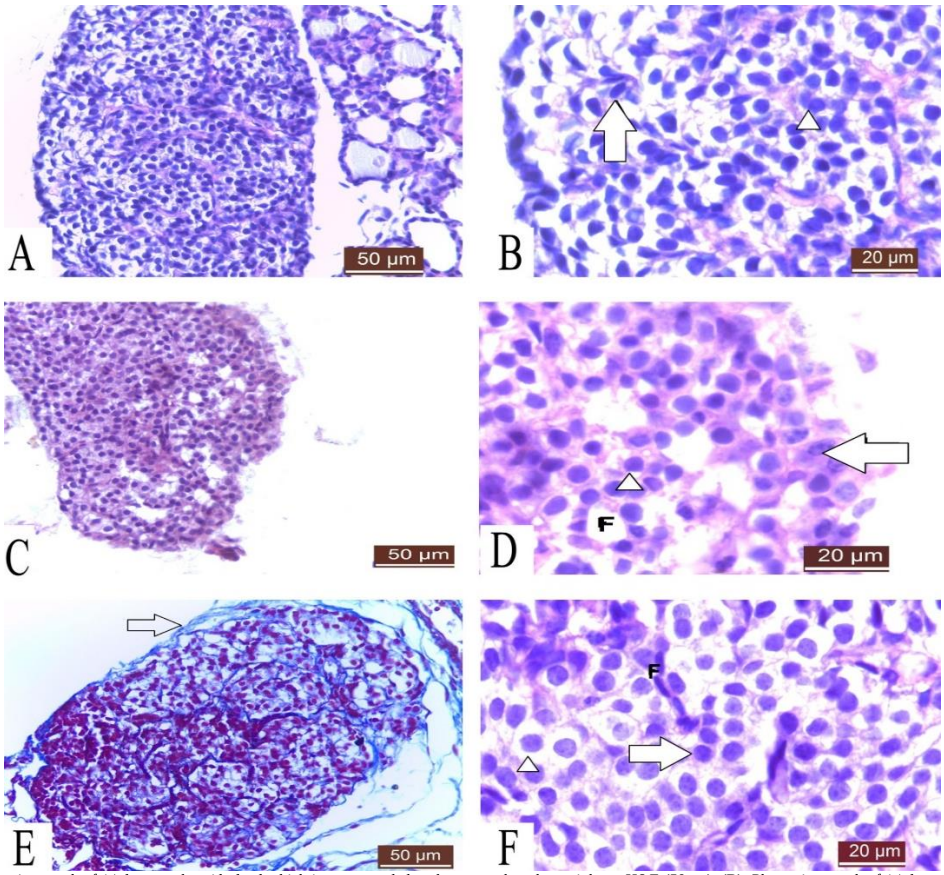

Figure 1: (A): Photomicrograph of 1st day parathyroid gland which is overcrowded at the center than the periphery. H&E (50µm). (B): Photomicrograph of 1st day parathyroid gland that contains oval cells with oval nucleus (arrow) and round cells with granular cytoplasm (arrowhead). H&E (20µm). (C): Photomicrograph of 2 weeks parathyroid gland showing cells form follicles at the periphery (arrowhead). H&E (50µm). (D): Photomicrograph of 2 weeks parathyroid gland showing highly dividing cells (arrow), follicles (F) and cells with vacuolated cytoplasm (arrowhead). H&E (20µm). (E): Photomicrograph showing fibrous connective tissue capsule (arrow). Masson trichrome (50µm). (F): Photomicrograph of 1-month parathyroid gland showing fibroblast (F) and round cells with empty (arrowhead) and granular cytoplasm (arrow). H&E (20µm).

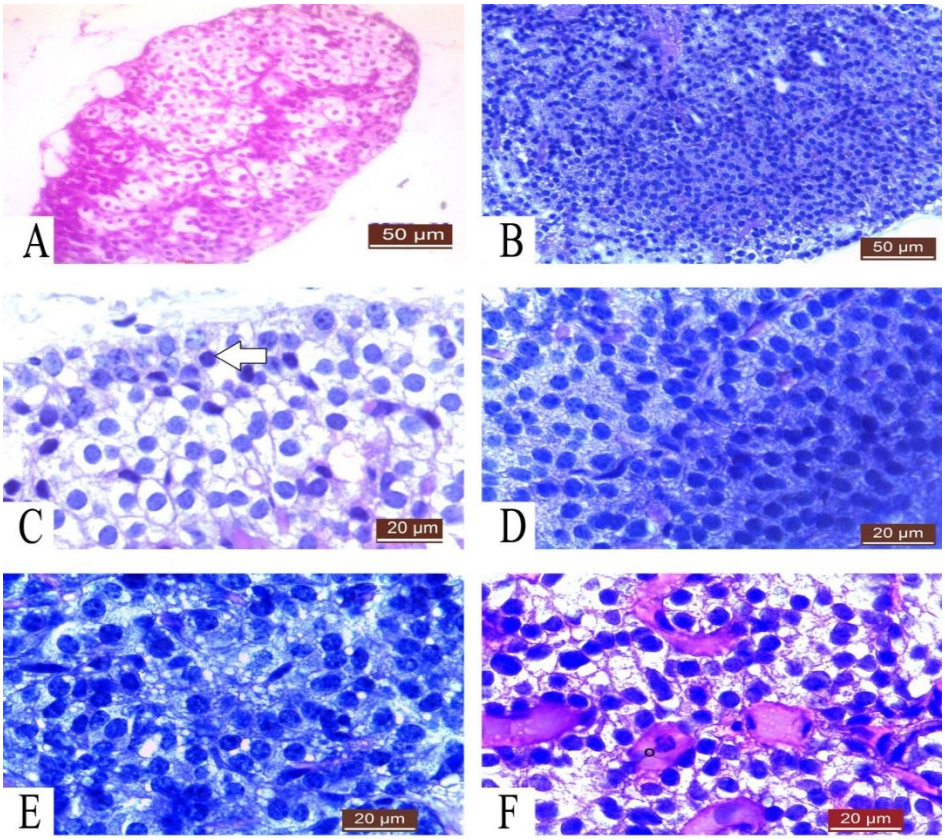

Figure 2: (A): Photomicrograph of 1-month parathyroid gland showing chief cells. PAS (50µm). (B): Photomicrograph of 10-month parathyroid gland becomes over crowded. H&E (50µm). (C): Photomicrograph of 4-month parathyroid gland showing lymphocytes with large nucleus occupy most of the cell with thin rim of cytoplasm (arrow). H&E (20µm). (D): Photomicrograph of 10-month parathyroid gland contains round cells with dark and light nuclei and granular cytoplasm. H&E (20µm). (E): Photomicrograph of 12months parathyroid gland showing vacuolated cytoplasm of most of cells. H&E (20µm). (F): Photomicrograph of 18months parathyroid gland showing few numbers of oxyphil cells (O). H&E (20µm).

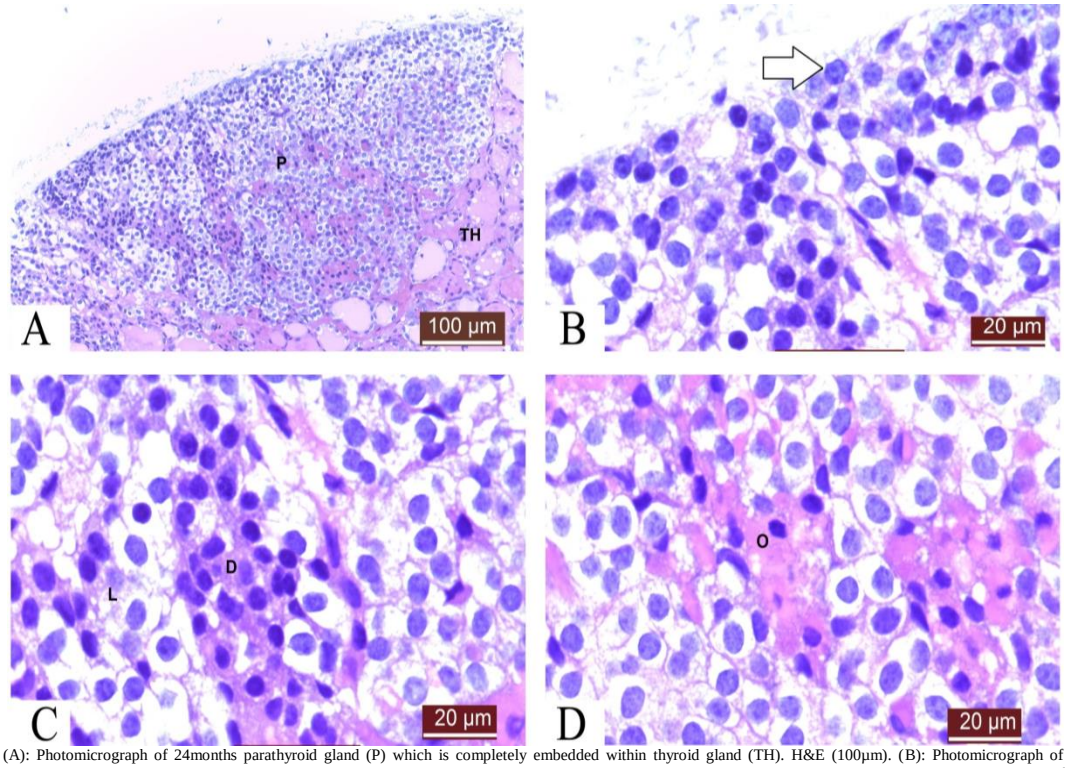

Figure 3: (A): Photomicrograph of 24months parathyroid gland (P) which is completely embedded within thyroid gland (TH). H&E (100µm). (B): Photomicrograph of 24 months parathyroid gland showing cells with prominent nucleolus (arrow) and cells with empty or vacuolated cytoplasm at the periphery. H&E (20µm). (C): Photomicrograph of 24 months parathyroid gland showing dark (D) and light (L) cytoplasm of chief cells. H&E (20µm). (D): Photomicrograph of 24 months parathyroid gland showing oxyphil cells (O) with acidophilic cytoplasm. H&E (20µm).

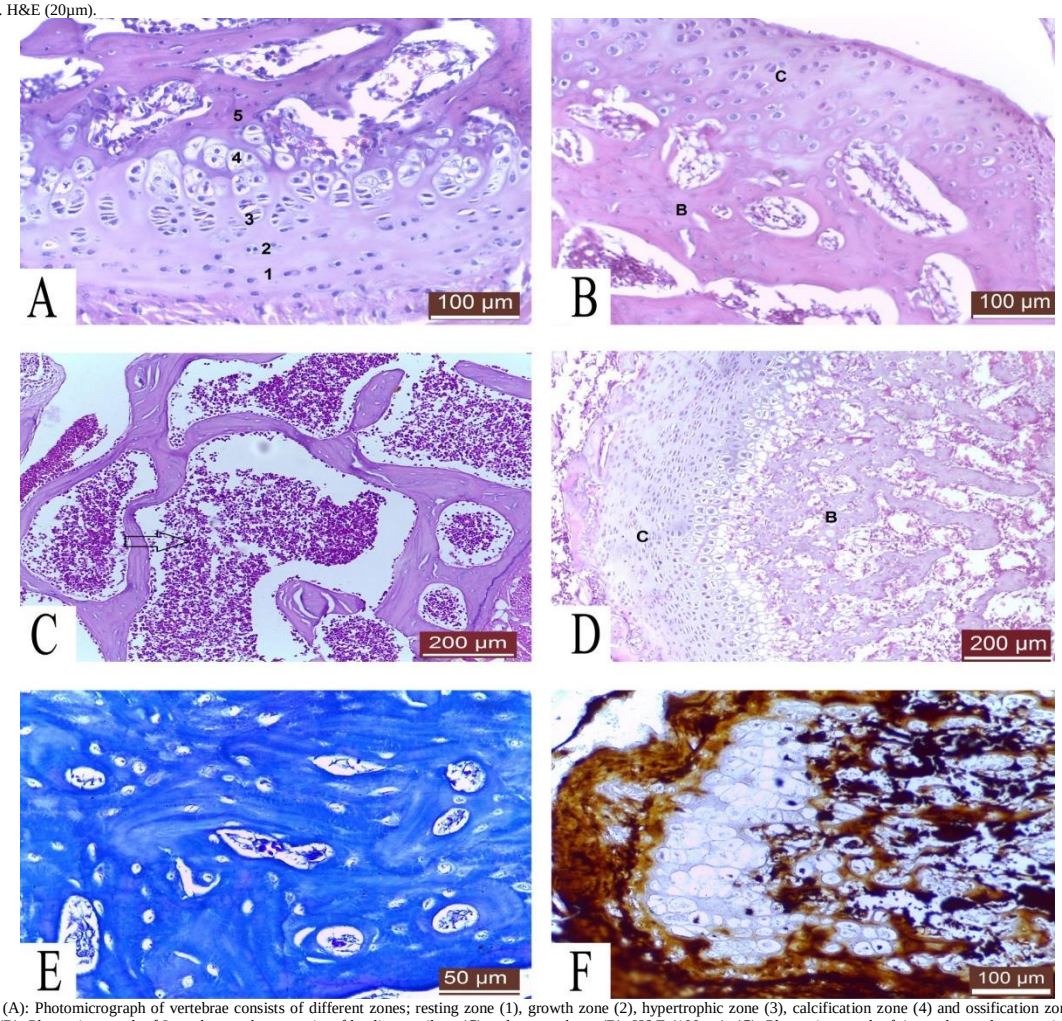

Figure 4: (A): Photomicrograph of vertebrae consists of different zones; resting zone (1), growth zone (2), hypertrophic zone (3), calcification zone (4) and ossification zone (5). H&E (100µm). (B): Photomicrograph of 2 weeks vertebrae consist of hyaline cartilage (C) and spongy bone (B). H&E (100µm). (C): Photomicrograph of 4-month vertebrae consists of spongy bone that contains marrow spaces (arrow). H&E (200µm). (D): Photomicrograph of 1 day femur consists of hyaline cartilage (C) and spongy bone (B). H&E (200µm). (E): Photomicrograph of 1 day femur showing bone lamellae. Masson trichrome (50µm). (F): Photomicrograph of 1 day femur showing black Calcium deposits. Von kossa (100µm).

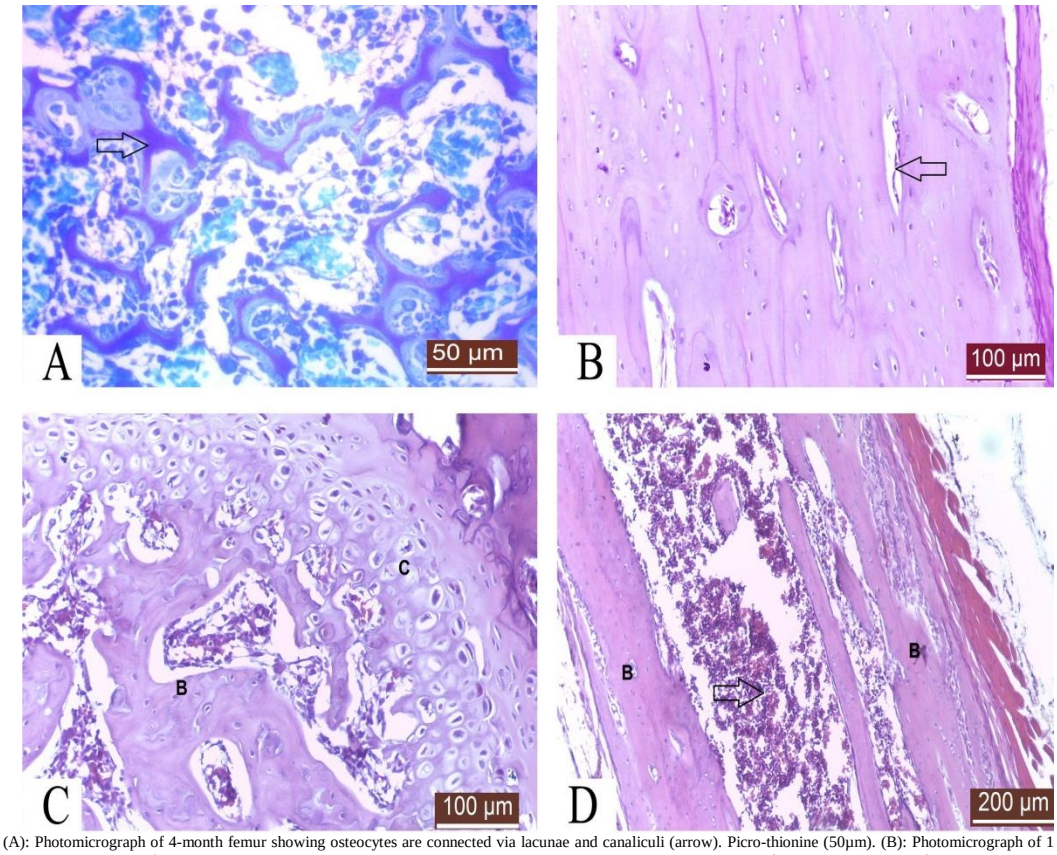

Figure 5: (A): Photomicrograph of 4-month femur showing osteocytes are connected via lacunae and canaliculi (arrow). Picro-thionine (50µm). (B): Photomicrograph of 1 month femur showing compact bone consists of Haversian system (arrow) with regular bone lamellae. H&E (100µm). (C): Photomicrograph of 2-weeks rib consists of hyaline cartilage (C) and spongy bone (B). H&E (100µm). (D): Photomicrograph of 1-month rib consists of compact bone (B) and medullary cavity (arrow).H&E (200µm).

4. DISCUSSION

The PTH is responsible for calcium homeostasis and maintenance of bone mineralization (Ramasamy, 2006).

Bone is considered the principal target organs of PTH. According to the secretory level of PTH, it has anabolic and catabolic effect on bone. The anabolic effect occurs through osteoblast activity and growth factors at high level of hormone (Esbrit and Alcaraz, 2013). The catabolic effect occurs with continuous secretion of PTH that stimulates osteoclast activity resulting in bone resorption and release of calcium and phosphate from the bone (Fuller *et al.*, 1998).

According to animal species, parathyroid gland is characterized by different location, lateral and anterior to the thyroid glands in Wister rat and separated by thick capsule in young animals (Birgit *et al.*, 1996) and lateral to and depressed within the thyroid gland in swiss mice (Jones *et al.*, 1983[b]). Guinea pig parathyroid gland was located at the lateral margin of thyroid gland at the middle third of thyroid gland. These results are similar to Swiss mice and Wister rats (Birgit *et al.*, 1996) and disagree with Syrian Hamster (Jones *et al.*, 1983[a]), human (Chen *et al.*, 2013) and swiss mice (Jones *et al.*, (b) 1983).

Guinea pig parathyroid gland consists of fibrous capsule and cellular parenchyma that composed of different cells and blood capillaries. These results were similar to parathyroid gland of mammals (Nagpal *et al.*, 1989; Ramayya *et al.*, 2012), rat, mice, and hamster (Basha and Wood, 1990).

Cellular components of parathyroid gland were chief and oxyphil cells. However, at young ages, it formed of only chief cells which appear irregular clumps, cords, and follicles around blood capillaries. These results were similar to results of human (Chen *et al.*, 2013; Mini and Manju, 2017) and not similar to results of rodents (Capen and Mohr, 1996; Nakanishi *et al.*, 2004).

Chief cells were polyhedral cells with round or oval nuclei. Cytoplasm of these cells showed different changes either granular or vacuolated and sometimes empty cells according to secretory phase of cells. The cells were firstly granulated as cytoplasm concentrated with PTH then became vacuolated when the hormone began to release. These results agreed with corresponding results of rat and mice (Birgit *et al.*, 1996; Enemali *et al.*, 2017) and human (Mini and Manju, 2017).

Guinea pig chief cells were differentiated into light and dark cells at late life stage which agreed with Wister rat and mice (Moreira *et al.*, 1985; Moreira and Goncalves, 1985). The dark chief cells were polyhedral shape with round nuclei. The nucleus was large and vesicular with peripheral heterochromatin. The dark cytoplasm was due to presence of several organelles and numerous secretory granules (Utsumi *et al.*, 1999). However, the light cytoplasm of light chief cells was present due to less cell organelles. These results are similar to results in camel (Al-Ramadan *et al.*, 2016) and human (Chen *et al.*, 2013).

There are no oxyphil cells in young guinea pigs which agree with rat and other lower animals (Cinti and Sbarbati, 1995). Oxyphil cells of Guinea pig parathyroid gland started to appear at 18 month of age and increase with age. These cells were larger than chief cells and characterized by more acidophilic cytoplasm. This result was in line with results of human and African giant rat (Enemali *et al.*, 2017). Numerous mitochondria in the cytoplasm were responsible for acidophilia of oxyphil cells that had a great role in energy production. This result was similar to those of mammals and human as oxyphil cells has a role in secretion of PTH with aging (Gerard, 2006).

Histologically, there were four cells forming the bone: osteogenic, osteoblast, osteocyte and osteoclast cells. Both type of bone; compact and spongy bones were formed of two layers, periosteum and endosteum. Periosteum composed of outer fibrous layer and the inner osteogenic layer. Endosteum consisted of osteocytes and calcified collagen fibers. These results were in line with human and rat bone (Bashandy and Elharoun, 2014; Ciocca *et al.*, 2015).

The bone formation rate was depended on number and activity of osteoblasts and osteocytes (Parfitt, 1990; Jilka *et al.*, 1998). Osteocytes were similar to osteoblast but present inside their lacunae with oval centrally located nuclei. These cells were surrounded by calcified matrix of bone. There were several cytoplasmic processes arise from osteocyte into the bone and forming canaliculi. The cytoplasmic processes of osteocytes were the main difference point between osteocyte and osteoblast cells. These processes and canaliculi had an important role in nourishment of osteocyte as they allowed passage of nutrients and fluids (Rehman *et al.*, 1994). Osteocytes were responsible for mobilizing the calcium from bone through their lysosomal activity and releasing of lytic enzymes (Sjastad *et al.*, 2010).

Many mammals and lower animals except Guinea pig characterized by presence of several Haversian systems in mature compact bone that is not present in prenatal bone of rat, human and pig (Broulik *et al.*, 1982). Guinea pig characterized by presence of Haversian vessels in compact bone with regular bone lamellae which similar to compact bone of rat (Bagi *et al.*, 1997).

Spongy bone was characterized by bone marrow spaces with irregular bone lamellae in between which similar to spongy bone of human, mice, and hamster (Bashandy and Elharoun, 2014). Spongy bone showed different changes with age, number, volume, and thickness of trabeculae are marginally decreased with age, while trabecular separation increased with age (Rehman *et al.*, 1994). Since the diameter of osteon and Haversian canal increased with age (Broulik *et al.*, 1982).

5. CONCLUSION

Guinea pig parathyroid gland characterized by appearance of oxyphil cells at 18 months and increased with age, while dark and light chief cells were begun to appear at 24 months. On the other hand, bone composed of both hyaline cartilage and bone at early postnatal life. Then bone completely ossified at different ages according to bone type. Haversian vessels were the main feature of Guinea pig compact bone instead of Haversian canals of mammals.

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