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A histological study on the prenatal development of the parathyroid gland of Guinea pig with relation to bone formation

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ABSTRACT

Parathyroid gland had a great importance in keeping of bone mineralization via the action of parathyroid hormone in maintaining the balance of serum calcium level. This study aimed to demonstrate the developmental histological structure of parathyroid gland and bone during prenatal life. The current study was done on twenty-nine Guinea pig embryos. Samples were collected from parathyroid gland and bone of different ages. The parathyroid gland of twenty-five embryonic days (ED) was originated from third pharyngeal pouch as endodermal thickening from the wall of pouch then at 27 ED appeared as a few polyhedral cells. At 30 ED, parathyroid gland appeared as single pair small condensation of cells close to trachea, thyroid gland, and thymus. At 65 ED, the cells of parathyroid gland showed different cytoplasmic changes according to hormone secretion. At early prenatal stage, femur and vertebrae composed of both cartilage and bone. At 65 ED, femur became formed of spongy bone. While ribs were consisted of hyaline cartilage only at the early prenatal stage, then became ossified at late prenatal stage, 65 ED.

1. INTRODUCTION

Guinea pig (*Cavia porcellus*) is one member of family Caviidae of rodents (Burnie, 2008).They are used as a source of food; as pets and for medical research (Morales, 1995; Terril and Clemons, 1997 and Gad, 2013). According to Nagpal *et al.*, (1989) and Ramayya *et al.*, (2012), the histological structure of parathyroid gland of Guinea pig is similar to that of mammals. Thus, studying the histology of parathyroid gland of Guinea pig will be a good indicator for other animals. The parathyroid hormone (PTH) has a role in calcium homeostasis. Muscle contraction, bone mineralization and blood coagulation depend on blood calcium level (Carafoli, 2003 and Ramasamy, 2006). PTH is considered the principal target organ of bone. PTH level control the bone mineralization, it has anabolic and catabolic effect (Fuller *et al.*, 1998 and Esbrit and Alcaraz, 2013).

Most of mammals have two pairs of parathyroid glands that originate from the third and fourth pharyngeal pouches (Isono *et al.*, 1985; Sheffield 2002; Prospero *et al.*, 2009 and Carlson 2010). However, Guinea pig has one pair of parathyroid glands originating from third pharyngeal pouch which begins to appear at 25 day of gestation period. At 27 embryonic day (ED) and upwards, parathyroid gland originate from the third pharyngeal pouch is embedded in the outer surface of the lateral lobe of the thyroid gland. Also, ossification of the fetal Guinea-pig skeleton begins at about 27 ED (Graham and Scothorne, 1971).

Therefore, the aim of this work is to throw more light on the developmental histological structure of parathyroid gland and bone during different prenatal stages of Guinea pig.

2. MATERIAL AND METHODS

The present study was carried out on 29 embryos of Guinea pigs brought into the Department of Histology, Faculty of Veterinary Medicine, Benha University, Egypt.

2.1. Animals and sampling

Guinea pig with 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 abnormal disease.

The pregnant Guinea pigs were euthanized to take the embryos. The number of specimen collected from animals at 25, 27, 30, 40, 50 and 65 ED were 5, 5, 5, 5, 5, and 4, respectively. The specimens were taken from parathyroid gland and bone.

The bone specimens were taken from femur, ribs, and vertebra. At early pregnancy, the young embryos were used as a whole in routine histological techniques. However, at the late pregnancy, opening at the neck region and separating the thyroid and parathyroid gland from the middle third of tracheal rings was done for the large embryos. Also, different bone samples; femur, ribs and vertebrae were obtained by opening the large embryos from the vent till thorax.

2.2. Light microscopy

The parathyroid specimens were fixed in 10% neutral buffered formalin for 48-72 hours, dehydrated in ascending

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grades of alcohol, cleared in xylene, and embedded in paraffin wax.

The bone specimens were fixed in 10% neutral buffered formalin for 48-72 hours, and then decalcified with decalcifying agents, 5% Hydrochloric acid. Decalcification occurred at 4°C with continuous shaking and continuous renewal of decalcifying agent daily till the bone become 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.

All Sections of 5 µm thickness were cut and stained, according to Bancroft and Gamble (2008).

The following stains were used:

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

3. RESULTS

3.1. Parathyroid gland

The primordium of the parathyroid gland appears as a thickening in the endodermal wall of the 3rd pharyngeal pouch at 25 ED (Fig.1A). It projects laterally to be surrounded by the mesenchymal cells between the 3rd and the 4th pharyngeal arches. There are several endodermal cells with acidophilic cytoplasm at 27 ED (Fig.1B).

At 30 ED, single pair of parathyroid gland locates between thyroid gland and thymus (Fig. 1C). It is surrounded by thin fibrous capsule consisting of collagen fibers and send fibrous septa into the parenchyma of gland (Fig.1D).Parathyroid gland consists of condensed chief cells (Fig.1E). Cells arrange randomly and some arranged in form of cords. Cells are spherical in shape, and some are oval shape with basophilic cytoplasm (Fig.1F).

They are PAS positive cells (Fig.2A). Also, there are other types of cells in the parenchyma of the gland, several fibroblasts which are oval in shape with oval nucleus (Fig.2B).

Later, at 40 ED, parathyroid gland descends close to trachea and thyroid glands owing to migration of thymus (Fig.2C). Parathyroid gland becomes more condensed with chief cells (Fig.2D). At 50 ED, some of cells form follicles. Parathyroid gland contains round and oval chief cells with dark and light nucleus with granular and vacuolated basophilic cytoplasm (Fig.2E).

Immediately before birth, at 65 ED, parathyroid gland contains several forms of cells. Most of cells are round cells with round nucleus but, few numbers of cells are oval with oval dark nucleus. Cytoplasm of cells may be granular, vacuolated, or empty cytoplasm (Fig.2F).

3.2. Bone

At 30 and 65 ED, both femur and vertebrae composed of cartilaginous and ossified parts (Figs.3A&B). The ossified part consists of spongy bone which is composed of numerous bone marrow cavities and irregular bone lamellae. Bone lamellae consist of calcified collagen fibers and osteocytes inside lacunae. These cells connect together via canaliculi (Fig.3C). At 30 ED, ribs are consisted of hyaline cartilage only (Fig.3D). Later, it becomes ossified at 65 ED (Fig.3E).

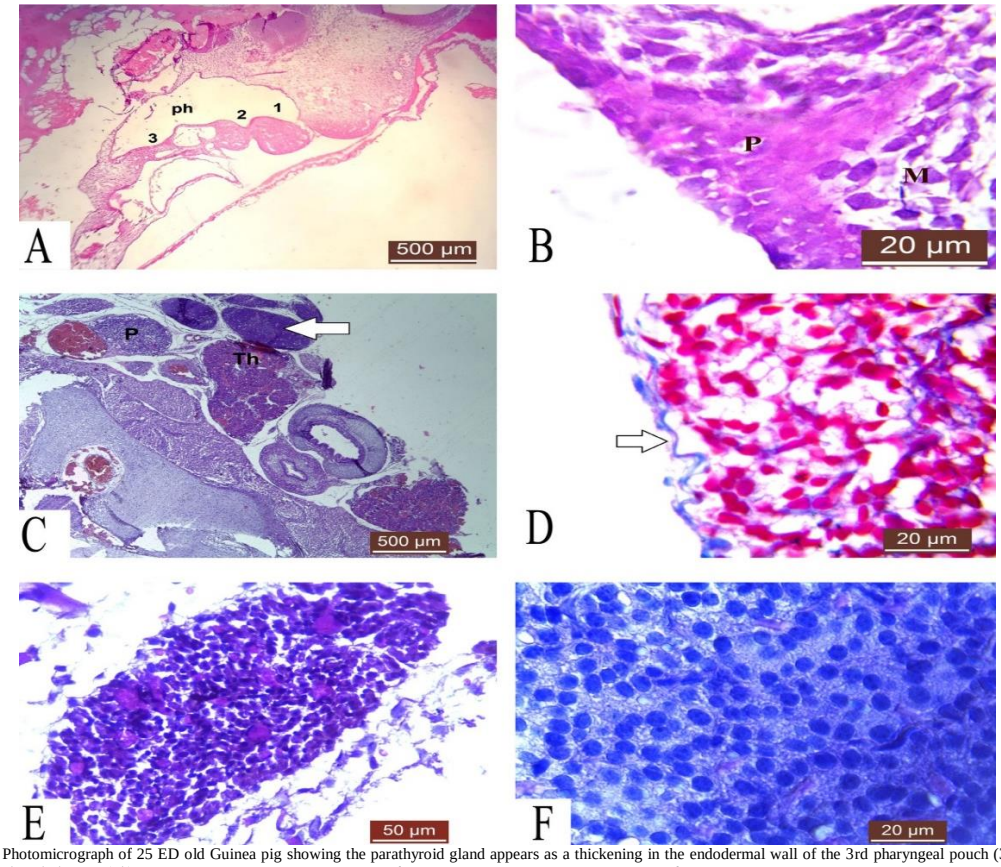

Figure 1. (A): Photomicrograph of 25 ED old Guinea pig showing the parathyroid gland appears as a thickening in the endodermal wall of the 3rd pharyngeal pouch (3), 1st (1), 2nd (2) pharyngeal pouch and pharynx (Ph). H&E, 500 µm. (B): Photomicrograph of 27 ED Guinea pig showing primordium of the parathyroid gland (p) and surrounding mesenchymal cells (M). H&E, 20 µm. (C): Photomicrograph showing parathyroid glands (P) of 30 ED are located close to trachea, thyroid glands (Th) and thymus (arrow). H&E (500µm). (D): Photomicrograph of parathyroid gland of 30 ED showing fibrous connective tissue capsule (arrow). Masson trichrome (50µm). (E): Photomicrograph of parathyroid gland of 30 ED showing condensed chief cells. H&E (50µm). (F): Photomicrograph of parathyroid gland of 30 ED showing cells arranged randomly and some arranged in form of cords. H&E (20µm).

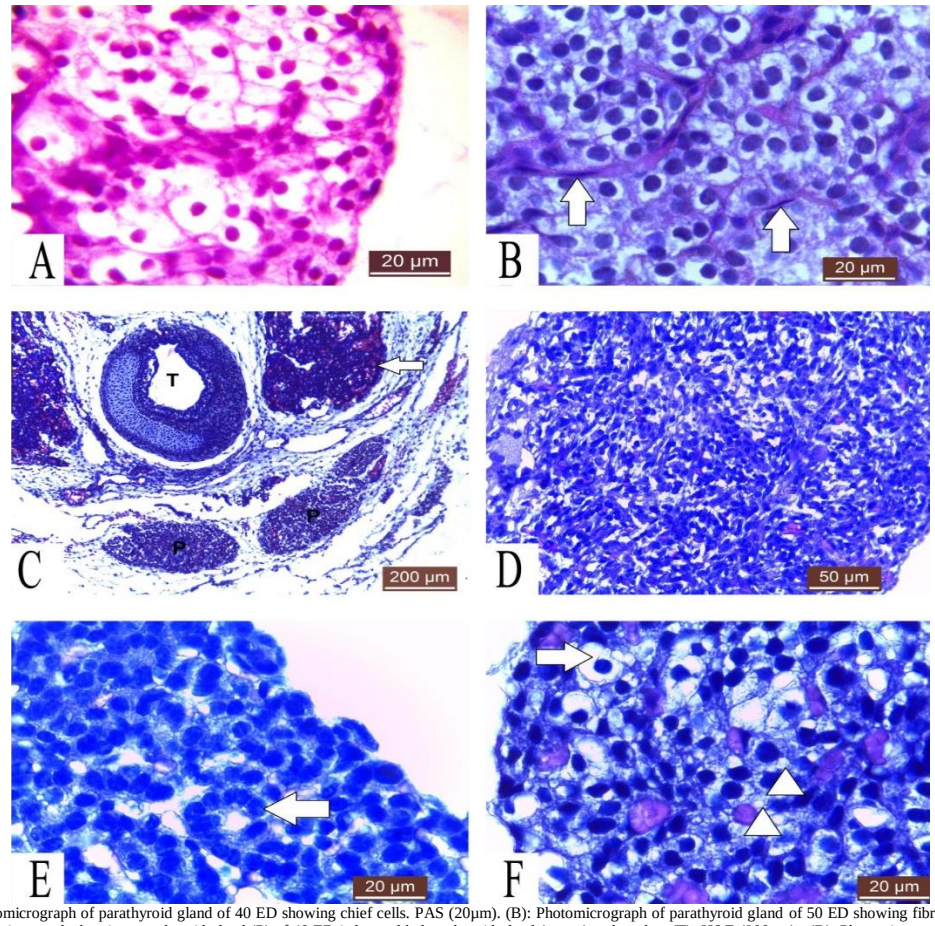

Figure 2: (A): Photomicrograph of parathyroid gland of 40 ED showing chief cells. PAS (20µm). (B): Photomicrograph of parathyroid gland of 50 ED showing fibroblasts (arrow). H&E (50µm). (C): Photomicrograph showing parathyroid gland (P) of 40 ED is located below thyroid gland (arrow) and trachea (T). H&E (200µm). (D): Photomicrograph of 40 ED showing very condensed parathyroid gland. H&E (50µm). (E): Photomicrograph of parathyroid gland of 50 ED showing some of cells form follicles (arrow) and chief cells contain granular and vacuolated basophilic cytoplasm. H&E (20µm). (F): Photomicrograph of parathyroid gland of 65 ED showing granular, vacuolated (arrowhead) and empty (arrow) cytoplasm. (20 µm).

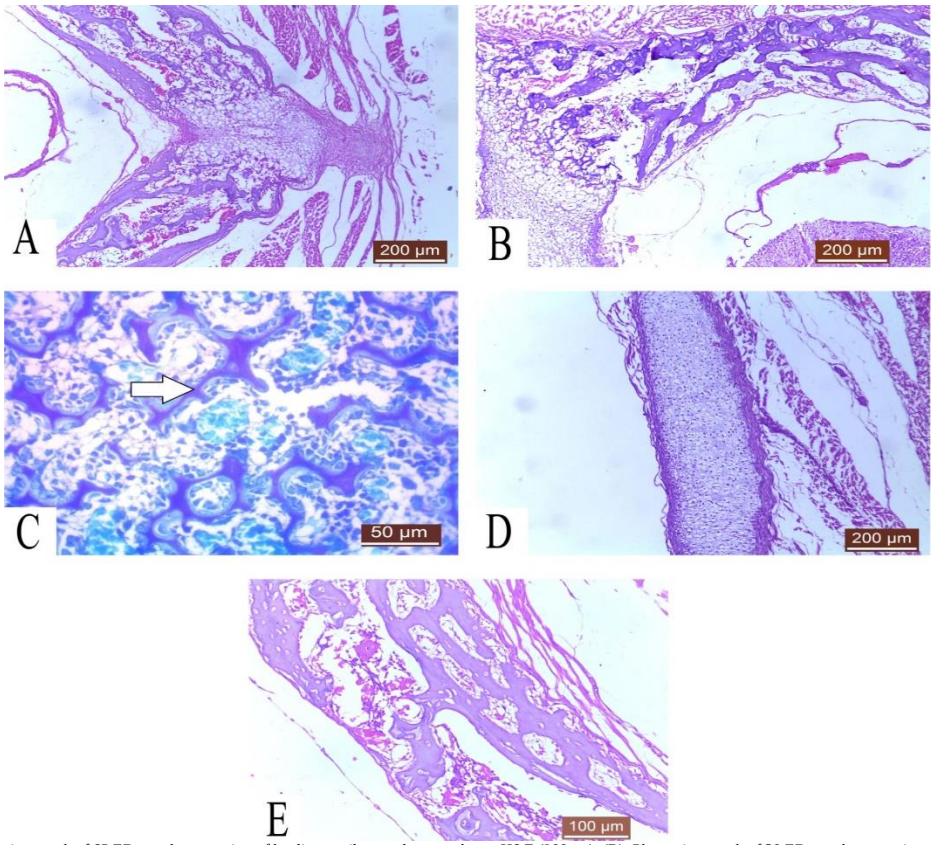

Figure 3: (A): Photomicrograph of 65 ED vertebrae consists of hyaline cartilage and spongy bone. H&E (200µm). (B): Photomicrograph of 30 ED vertebrae consists of hyaline cartilage and spongy bone. H&E (200µm). (C): Photomicrograph of 30 ED vertebrae showing osteocytes are connected via lacunae and canaliculi (arrow). Picro-thionine (50µm). (D): Photomicrograph of 30 ED rib consists of hyaline cartilage only. H&E (200µm). (E): Photomicrograph of 65 ED rib consists of spongy bone. H&E (50µm).

4. DISCUSSION

Guinea pig parathyroid gland is found at the lateral margin of thyroid gland at the middle third of thyroid gland. This result is agreeing with parathyroid gland of Wister rat (Birgit *et al.*, 1996) and disagrees with Syrian Hamster, mice, and African giant rat (Jones et al., 1983[a,b,c]; Enemali *et al.*, 2017).

Only one pair of parathyroid gland originates from third pharyngeal pouches in guinea pig, which is similar to the corresponding ones of Golden Hamster (Isono et al., 1985), Wister rats (Birgit et al., 1996), and mice (Chen *et al*., 2013). On the other hand, African giant rat, pig, rabbit, and most mammals have four parathyroid glands arise from third and fourth pharyngeal pouches (Prospero *et al.*, 2009; Enemali *et al.*, 2017).

Parathyroid gland of Guinea pig begins to appear at 25 ED as endodermal thickening from the third pharyngeal pouch and at 27ED appear as cluster of cells only. This result is in line with results of Graham and Scothorne (1971). On the hand, human primordial appears at fourth week of embryonic life (Gardner and Shoback, 2011).

Sheffield (2002), Moore-Scott and Manley (2005) and Carlson (2010) demonstrated the mice parathyroid glands development; they begin to develop with the thymus from the 3rd pharyngeal pouch at 8 ED. The 3rd pharyngeal pouches become visible at 9-10 ED. The parathyroid and thymus can be recognized at 12-13 ED. Later the parathyroid glands separate from the thymus at 14-15 ED. Finally, the parathyroid glands locate posterior to the thyroid gland, while the thymus descends in the direction of the heart (Blackburn and Manley, 2004).

Histologically, parathyroid gland of Guinea pig is similar to parathyroid gland of mammals (Ramayya *et al.*, 2012), rat and hamster (Basha and Wood, 1990). At prenatal life, parathyroid gland consists mainly of chief cells which arrange in form of irregular cords and follicles. There is no differentiation of chief cell into dark and light cells. These results are similar to those of human (Chen *et al.*, 2013; Mini and Manju, 2017), Wister rat and mice (Jones *et al*, 1983[b,c]; Moreira *et al.*, 1985; Moreira and Goncalves, 1985).

Chief cells are polygonal cells with spherical or oval dark and light nuclei. These cells are characterized by different cytoplasmic changes either granular or vacuolated and sometimes empty cytoplasm according to secretory phase of cells. These results agree with corresponding results of rat and mice (Birgit *et al.*, 1996; Enemali *et al.*, 2017) and human (Mini and Manju, 2017).

PTH has a great effect on bone mineralization. At high levels, PTH has an anabolic effect in the bone through stimulation of osteoblast activity and growth factors (Hock and Gera, 1992; Esbrit and Alcaraz, 2013). While continuously high levels PTH stimulate osteoclast activity leading to bone resorption and release of boney minerals. The PTH prevents apoptosis of mature osteoblasts, so it increases its lifespan (Dempster *et al.*, 1993; Weinstein *et al.*, 1998).

Bone is considered as a hard connective tissue as it contains minerals in form of calcium and phosphorus insoluble salts represent sixty-five percent of boney tissue and bound to the organic matrix giving the bone hardness (Ciocca *et al.*, 2015; Dermience *et al.*, 2015).

Bone begins to develop at 27day gestation period of Guinea pig that similar to results of Grahum and Scothorne (1971). However, rat bone starts to develop at 21-day gestation period (Baiera and Viertel, 2019). At early prenatal life, Bone is formed from cartilage then become calcified during late stage of pregnancy and continuous till puberty and forms the main skeleton of older animal to withstand the great forces than young animals (Sjastad *et al.*, 2010). Long and flat bones are undergoing endochondrial ossification where cartilage is replaced by bone (Scheuer *et al.*, 2000; Ross and Pawlina, 2011).So that, bone development differs according to age of the animal (Root *et al.*, 1997; Perry *et al.*, 2014).

5. CONCLUSION

Parathyroid gland of Guinea pig begins to develop at 25 ED from the third pharyngeal pouch and appear as single pair of glands. At 30 ED, it becomes surrounded by separate fibrous capsule and contains only chief cells. On the other hand, bone at early prenatal life consists of both hyaline cartilage and spongy bone then becomes completely ossified at 65ED. Therefore, further studies should be done on the parathyroid gland and bone of Guinea pig at prenatal life.

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