ABSTRACT

Bone is a hard and resilient tissue structure that provides mechanical support for the body of an organism. This study was aimed to evaluate the radiographical and biochemical changes during postnatal bone development of forelimbs in Nigerian indigenous puppies from 2-week-old to 24-week-old. Six (6) Nigerian indigenous puppies were chemically restrained using chlorpromazine at 2 mg/kg I.M. and positioned using ropes and sandbags for mediolateral and craniocaudal views of bones of the forelimbs. At 2-week-old, radiographic evaluation of bones of the forelimbs were undertaken and continued bi-weekly up to 24-week-old. Serum samples were analysed from 4-week-old to 24-week-old at four weeks' interval for mineral (electrolytes) using colometric method for calcium and phosphorus and flame photometry method for sodium and potassium. At 2-week-old, there was an ill-defined radiopaque secondary ossification centre at the proximal epiphysis and a small ovoid radiopaque structure at the distal epiphysis of the humerus. There was not any sign of secondary ossification centre at proximal and distal epiphyses of both radius and ulna. There was the presence of a small radiopaque (ill-defined) accessory carpal. 1st to 5th metacarpals were present together with the proximal, middle and distal phalanges of all digits except the 1st digit that has proximal and distal phalanges. From 20-week-old, fully developed carpals and bony structures were observed through 24-week-old where the commencement of fusion of apophyses of olecranon (proximal epiphysis of ulna) at the centre and acrophyses of metacarpals and phalanges. The mean values (mean ± SEM) from serum biochemistry of calcium, phosphate, sodium and potassium in mmol/L from this study were within the normal limits throughout the study. Conclusively, the complementary information from calcium, phosphate, sodium and potassium in this study showed that these elements are within the normal limit that suggests normal bone development during the study period, which stressed the importance of the knowledge on serial changes of bone development which could be useful in clinical practice.

Keywords: Carpals; Humerus; Metacarpals; Radius; Ulna
INTRODUCTION

Bone is a hard and resilient tissue structure that provides mechanical support for the body of an organism [1]. The composition of bone can be best described in heterogeneous phases made up of mineral, organic and water [2]. The mineral phase comprises majorly calcium and phosphorus, carbonate, magnesium and sodium [3]. The organic phase consists of type 1 collagen as the abundant protein in the bone matrix that provides elasticity to bone tissues, stabilize the extracellular bone matrix and support deposition of mineral. Non-collagenous proteins that form 5% total weight of the bone include small integrin-binding N-glycosylated (SIBLING), small leucine-rich proteoglycans (SLRP), g-carboxyglutamic acid protein (GLA protein) and small secreted cysteine-rich protein (CCN protein) that regulate mineralization. Lipids as fat-soluble forms less than 3% of the bone matrix surrounding cells, which control the flux of ions and, transmit and receive molecules in and out of the cell [2]. Water serves many functions, including filling the pores, interacting with collagen fibrils and binding to mineral crystals [4].

Bone tissue is made up of cells which include osteoblasts, that play a role in the formation (osteoid) and mineralization of bone tissue [5], osteocytes, and osteoclasts, which are associated with the reabsorption of bone tissue [6]. Osteoblasts and osteocytes originated from osteoprogenitor cells of mesenchymal origin, while osteoclasts originated from the same cells that metamorphosed to macrophages and monocytes [7]. There are also hematopoietic stem cells in the marrow of the bone that produce white blood cells, red blood cells, and platelets [8].

Bones are classified according to shape and or length which includes long bones, short bones, sesamoid bones, flat bones and irregular bones [9]. Bone development is closely associated with the activities of chondrocytes in a cartilaginous component called growth plate, which comprises the reserved zone, proliferative zone and hypertrophic zone [10,11,12,13], and the hypertrophic zone was further divided into maturation zone and calcification zone [10,11]. Factors such as nutrition, hormones and growth factors also play a significant role in longitudinal skeletal development [14].

There is a paucity of knowledge on the serial changes during bone growth of forelimbs in the Nigerian indigenous puppies. Only extrapolations or inferences have been made for Nigerian indigenous puppies from the knowledge of bones of the forelimbs of exotic breeds. Therefore, radiographic information of the developmental pattern of bones of the forelimbs in Nigerian indigenous puppies will assist in understanding developmental and radiologic anatomy, and bone disorders in the breed. This study aimed to evaluate the radiographical and biochemical changes during postnatal bone development of forelimbs in Nigerian indigenous puppies from 2-week-old to 24-week-old.

MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where the radiographical examinations were carried out in the Diagnostic Imaging Center, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. 3 male and 3 female puppies from 3 different Nigerian indigenous bitches were used for the study. Post whelping, these puppies were selected based on normal anatomical conformation and haematological parameters. Each puppy was tagged and numbered for easy identification to avoid mixing up data.

Nigerian indigenous puppies were chemically restrained using chlorpromazine at 2 mg/kg I.M. and positioned using ropes and sandbags for mediolateral and craniocaudal views of bones of the forelimbs. At 2-week-old, radiographic evaluation of bones of the forelimbs was undertaken and continued bi-weekly up to 24-week-old. Serum samples were evaluated from 4-week-old to 24-week-old at four weeks (4,8,12,16,20 and 24) interval for mineral (electrolytes) using colometric method for calcium and phosphorus [15] and flame photometry method for sodium and potassium [16] to explain the nature of bone metabolism or calcification during the period of study.
The focal film distance was set at 50 cm while the exposure factors were also set at the following Kilovolts (50-65), milliamperes (25) and milliamperes per second (3) for exposure of bones of the forelimbs using mobile x-ray machine (Recorders and Medicare systems (P) Ltd model MDX-100).

For radiographic exposure of bones of the forelimbs in a humane manner, mediolateral views were undertaken by casting the puppy on the right lateral recumbency and the lower forelimb was drawn cranially and secured, and then the upper forelimb was pulled caudally out of the way and also secured. The head and neck were extended and secured. The beam was centred on the elbow joint of the right forelimb and collimated from the shoulder joint to the phalanges then exposed. Cranio-caudal views were undertaken by casting the puppy on dorsal recumbency and ensured not tipped on either side. The left forelimbs were extended cranially while the right forelimbs were extended caudally, and the hindlimbs were also extended caudally and secured. The beam was centred on the elbow joint of the right forelimb and collimated from the shoulder joint to the phalanges then exposed.

Post radiographic exposure, the exposed films were immediately offloaded in the darkroom and developed for radiologic interpretation. Dried radiographs were placed on the radiographic viewer and observed keenly for the radiologic anatomical details of bones of the forelimbs.

RESULTS

At 2-week-old, there was an ill-defined radiopaque secondary ossification centre (SOC) at the proximal epiphysis and a small ovoid radiopaque structure at the distal epiphysis of the humerus. There was not any sign of SOC at proximal and distal epiphyses of both radius and ulna. There was the presence of a small radiopaque (ill-defined) accessory carpal. 1st to 5th metacarpals were present together with the proximal, middle and distal phalanges of all digits except the 1st digit that has proximal and distal phalanges (Figure 1). At 4-week-old, the ill-defined SOC at proximal and distal epiphyses transformed to well-defined epiphyses with an opened growth that separated them from their metaphyses. There were ill-defined SOC at proximal and distal epiphyses of radius, but no sign of SOC at proximal and distal ulna. There was the presence of ill-defined radial, ulnar and developing accessory carpal, 1st to 4th carpals. Metacarpals and phalanges became more calcified with the presence of acrophyses at distal segments of metacarpals and proximal segments of proximal, middle, and distal phalanges of all digits. At 6-week-old, well-defined SOC at proximal and distal epiphyses of humerus continue developing. There was well-defined SOC at proximal and distal epiphyses of radius, but there was not a sign of SOC at the proximal and distal of the ulna. Carpals were simultaneously developing. Metacarpals and phalanges were also further developing (Figure 2). At 8-week-old, humerus and radius were gradually developing with their growth plates becoming narrower, and there was the presence of ill-defined SOC at the distal epiphysis of the ulna. Carpals were still developing. Metacarpals and phalanges were developing with their acrophyses becoming narrower (Figure 3). At 10-week-old, well-defined SOC at distal epiphysis of the ulna and well-defined accessory carpal were observed (Figure 4). At 12-week-old, every bone structure on the forelimbs was simultaneously developing and the appearance of a small radiopaque as SOC at proximal epiphysis of the ulna. From the 14-week-old, there was an ill-defined proximal epiphysis of the ulna (olecranon) and continues development of every bone structure that extends through 16-week-old to 18-week-old where well-defined olecranon was observed. From 20-week-old, fully developed carpals and advanced development of bone structures were observed. This continue through weeks 22 to 24 old where the commencement of fusion of apophyses of olecranon at the centre (Figure 5) and acrophyses of metacarpals and phalanges have fused (Figure 6).

The mean values (mean ± SEM) of calcium, phosphate, sodium and potassium in mmol/L from this study were obtained and represented in Table 1.
DISCUSSION
In this study, some important information about the serial changes in skeletal development of forelimb during the six months of age, in medium-sized Nigerian indigenous dogs was provided. The radiographic findings from the postnatal skeletal development of the forelimb in Nigerian indigenous dogs were similar to that reported by [17] and [18] except for the humerus, which presents the appearance of SOC at the proximal and distal epiphyses at 4-week-old unlike the reported studies that appeared from 8-week-old and 3-week-old respectively. However, Von Dfeil and DeCamp [19] stated that the SOC at proximal and distal epiphyses of humerus and radius appeared at prenatal, while the SOC of the ulna, carpals, metacarpals and phalanges appeared from 3 to 4 months of age. This differences could be attributed to the fact that, the number, location, and time of appearance of SOC differs between breeds, and even within breeds, variation in the time of fusion still exist [20].

The development of secondary ossification centres at epiphyses brings about the complete appearances of growth plates [21]. The secondary ossification centres first appeared as tiny (ellipsoid) radio-opaque area resembling a drop of calcium with no well-defined borders, which develop into an oval-shaped (lunate) radiopaque structure with still no defined borders then further developed into a prominent epiphysis with well-defined borders that is differentiated from the metaphysis by a radiolucent growth plate [22]. The rate of bone development in early life occurred with the activity of chondrocytes [23] in the cartilaginous component of the growth plate, composed of the reserved zone, proliferative zone and hypertrophic zone [10,11,12,13], where the hypertrophic zone is further divided into maturation zone and calcification zone [10,11]. The reserved zone contains stem-like chondrocytes which produce clones of proliferative chondrocytes and a morphogen that organized the proliferative replicants [24]. The proliferative zone is the zone of active mitotic replication of chondrocyte into daughter chondrocytes that are organized into columns of its axis. In the hypertrophic zone, the chondrocytes enlarge to become the maturation zone [25], which then degenerates to become mineralized, forming the calcification zone [26].

The changes that occurred were due to high metabolic activities influenced by nutrition leading to an increased rate of bone development [27]. The mineral analyses of electrolytes such as calcium, phosphate, sodium and potassium in this study showed that these elements are within the normal limit that concur with Bush [28], which suggests normal bone development during the study period as stated by Von Pfeil et al. [29]. The bones stored about 99% of the calcium in the body which offers structural support for the body and also serves as a reservoir in calcium homeostasis [30]. It is regulated in the body by parathyroid hormone (PTH), calcitonin and vitamin D [31]. Bone is dynamic especially during the growing phase of an animal because there is a higher rate of bone formation than bone resorption. After all, calcium is being utilised for rapid bone mineralization [32]. High sodium alters calcium metabolism by increasing urinary calcium excretion (calciuria), which may lead to an increase in the rate of bone resorption [33].

Phosphorus is an inorganic constituent of a bone, which is the sixth most abundant element in the body [34]. Phosphorus and calcium are the two main ionic elements required for hydroxyapatite formation during bone mineralization [35]. Phosphorus has a high influence on the activities of osteoblasts and osteocytes in the process of bone mineralization [36]. There is a strong relationship between sodium and calcium [37]. Potassium is also known as the hidden bone guardian, due to the role it plays along with sodium in maintaining critical fluid balance, but its influence on bone health is neglected. However, it promotes certain alkalinizing potassium compounds to neutralize the bone-depleting acids produced during metabolic processes [38]. By maintaining the acid-alkaline balance in the bodies, potassium prevents calcium lost in urine [39].

Conclusively, the data obtained from this study demonstrated important information about postnatal bone development in Nigerian indigenous puppies. The pattern and periodic characteristics of the growth plate and SOC
were defined. This can be able to support ageing in the breed through the serial appearance of their features over the time of the study. Calcium, phosphate, sodium and potassium analyses were within normal range throughout the study and this support the fact that the serial bone development in this breed adhere to standard. Finally, this study stressed the importance of the knowledge on serial changes of bone development which could be useful in clinical practice.

Acknowledgement
I appreciate the efforts of the entire technical staffs of Diagnostic Imaging Center, Ahmadu Bello University, Zaria.

REFERENCES


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### Table 1: Biochemical evaluation of the Nigerian indigenous puppies used in this study.

<table>
<thead>
<tr>
<th>Age</th>
<th>Ca (mmol/L) Range</th>
<th>Mean ± SEM</th>
<th>PO4 (mmol/L) Range</th>
<th>Mean ± SEM</th>
<th>Na (mmol/L) Range</th>
<th>Mean ± SEM</th>
<th>K (mmol/L) Range</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-week-old</td>
<td>2.6 - 2.9</td>
<td>2.7 ± 0.047</td>
<td>1.1 - 1.4</td>
<td>1.3 ± 0.041</td>
<td>139 – 145</td>
<td>142 ± 2.4</td>
<td>4.1 - 4.9</td>
<td>4.4 ± 0.16</td>
</tr>
<tr>
<td>8-week-old</td>
<td>2.4 - 2.8</td>
<td>2.7 ± 0.064</td>
<td>1.1 - 1.4</td>
<td>1.2 ± 0.049</td>
<td>145 – 155</td>
<td>147 ± 0.0</td>
<td>4.6 - 4.6</td>
<td>4.6 ± 0.0</td>
</tr>
<tr>
<td>12-week-old</td>
<td>2.4 - 2.6</td>
<td>2.5 ± 0.040</td>
<td>0.98 - 1.2</td>
<td>1.1 ± 0.035</td>
<td>139 – 153</td>
<td>143 ± 2.9</td>
<td>4.4 - 5.2</td>
<td>4.6 ± 0.16</td>
</tr>
<tr>
<td>16-week-old</td>
<td>2.4 - 2.9</td>
<td>2.5 ± 0.082</td>
<td>0.98 - 1.2</td>
<td>1.1 ± 0.046</td>
<td>142 – 147</td>
<td>144 ± 3.6</td>
<td>5.2 - 5.7</td>
<td>5.4 ± 0.076</td>
</tr>
<tr>
<td>20-week-old</td>
<td>2.4 - 2.7</td>
<td>2.6 ± 0.044</td>
<td>1.0 - 1.6</td>
<td>1.5 ± 0.16</td>
<td>149 – 150</td>
<td>150 ± 2.3</td>
<td>4.7 - 5.1</td>
<td>4.9 ± 0.056</td>
</tr>
<tr>
<td>24-week-old</td>
<td>2.4 - 2.6</td>
<td>2.5 ± 0.017</td>
<td>1.1 - 1.6</td>
<td>1.5 ± 0.13</td>
<td>138 – 154</td>
<td>143 ± 1.8</td>
<td>4.7 - 5.3</td>
<td>5.1 ± 0.10</td>
</tr>
<tr>
<td>Reference values*</td>
<td>2.0 – 3.0</td>
<td>0.8 - 1.6</td>
<td>140 – 155</td>
<td>3.6 - 5.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reference values*: Bush [28]
Figure 1: Demonstrating the ill-defined proximal and distal epiphyses of humerus, and ulnar carpal at 2-week-old

Figure 2: Demonstrating the carpals, metacarpals and phalanges of digits at 6-week-old
Figure 3: Demonstrating the developing carpals, metacarpals and phalanges at 8-week-old

Figure 4: Demonstrating the continues development of the forelimb bones at 10-week-old
Figure 5: Demonstrating the commencement of fusion of olecranon’s apophysis at 24-week-old

Figure 6: Demonstrating the fusion of acrophones of metacarpals and phalanges at 24-week-old