Clinical, Biochemical and Radiographic Alterations in Kittens with Experimental Induced Nutritional Secondary Hyperparathyroidism

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A B S T R A C T

Nutritional secondary hyperparathyroidism (NSHPT) is a metabolic disorder that is caused by excessive phosphate in diets, insufficient calcium, or both. This study was carried out on 20 male kittens with 2-3 month of age. The NSHPT was induced in 10 kittens by feeding solely on heart beef meat for 9 weeks. The other 10 kittens were fed on commercial diet as control. Clinically, affected kittens were unable to stand or walk then recumbent with bone abnormalities lameness and lately loss their body weight. Serum biochemical changes showed significant (P<0.05) increase in CPK, ALP, phosphorus and 1,25 dihydroxyVit.D3, but showed significant (P<0.05) decrease in 25(OH)Vit.D3, total protein, albumin, globulin, blood urea nitrogen, creatinine, calcium, magnesium, copper and zinc in cats with induced NSHPT compared to control.Radiographic changes in cats with induced NSHPT showed generalized decrease in bone opacity which affects entire skeleton (osteoporosis); cortices become thin and faint with decreased bone to soft tissue contrast. It was concluded that NSHPT is associated with clinical, biochemical and radiographic changes in young growing kittens. It is recommended to give kittens a balanced diet especially in Ca and P during the first 9 weeks of life.

Keywords: Kittens, NSHPT, Radiography.

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1. INTRODUCTION:

The parathyroid glands in cats consist of four small oval disks in the neck located near the thyroid gland (Rijnberk and Kooistra 2010). The parathyroid glands produce the parathyroid hormone (PTH), that plays a key role for maintaining calcium, phosphorus and vitamin D homeostasis and bone health **(**Hindié *et al.,* 2009**).**Hyperparathyroidism is over-activity of the parathyroid glands resulting in excessive production of parathyroid hormone (PTH) **(Rasor *et al., 2007*).** Hyperparathyroidism is due to an increase in PTH secretion which may be primary due to neoplastic transformation of the parathyroid gland (PHPT) or secondary due to a nutritional imbalance (NSHPT), or renal disease (RSHPT) (Adin., 2011). The typical clinical signs of NSHPT in kittens include lameness, reluctance to stand or walk and skeletal pain. Swollen costochondral

junctions and metaphyses, severe osteopenia and multiple fractures of long bones, scapulae, pelvis nasal bone or spine can follow relatively mild trauma and constipation (Moarrabi *et al.,* 2008). The serum biochemical profile usually associated with changes in serum calcium, creatinine, magnesium, total protein (TP), phosphorus, serum creatinine phosphokinase, blood urea nitrogen and parathyroid hormone concentration in a case of cats with NSHPT (Nagata and Yuki ,2013). Cats with NSHPT showed abnormal levels of different forms of vitamin D3 and alkaline phosphatase. (Parker *et al.*, 2015). Secondary hyperparathyroidism is usually related to trace element disturbance especially calcium, phosphorus, copper, magnesium and zinc (Khan *et al.,* 2005 and Pedram *et al.,* 2014).

This study aimed to monitor the clinical, serum biochemical and radiographic changes associated with induced NSHPT in kittens.

2. MATERIALS AND METHODS:

2.1. Animals and experimental design

The present study was carried out on twenty apparently healthy male kittens aged between 2 to 3 months and weighted between 0.45-0.85 kg. The kittens were randomized classified into two groups. Group I: included ten male kittens that were used as a control. The kittens in this group were fed on commercial balanced diet (HAPPY CAT-Grain free junior © Happy cat –Premium Cat Food-Germany) with recommended daily amount as described by the supplier (50gm. / 2 months cat / day) and tap water. Group II: including ten male kittens that were experimentally induced with NSHPT by feeding diet of beef heart that contains high P and low Ca concentrations. In addition, iodine was added to all kittens of Group2 (150 µg/cat/day) and with free access to tap water.

2.2. Clinical examination

The kittens were subjected to clinical examination and all clinical signs were recorded as previously described (Kelly, 1984).

2.3. Biochemical examination

Special chemical kits (Produced by Bio-analytic Company, Egypt) were used for determination of parathyroid (PTH), 25-hydroxyvitamin D3, 1, 25-dihydroxy vitamin D3, urea (BUN), creatinine, creatinine phosphokinase (CPK), alkaline phosphatase (ALP), total Calcium (Ca), phosphorus, copper (Cu), zinc (Zn) and magnesium (Mg), total protein (TP), albumin (Alb) and globulin (glob) and Alb/glop (A/G) ratio.

2.4. Radiographic examination.

Radiography examination of the entire skeleton was performed by using 100 KV and 80 mA X-ray machines (Mobile HP), 80 cm FFD, 55KV and 4.5 mA., according to the method described by Bassert and McCurnin (2010).

2.5. Statistical analysis

The mean values of diseased groups at different time periods were compared at different periods and control group using one-way analysis of variance (ANOVA). The values were considered significant at $P<0.05$.

3. RESULTS:

3.1. The clinical signs:

Clinical signs were first observed during the fourth week in the experimentally affected kittens. These signs included sudden onset of posterior lameness of various intensity and disinclination (reluctance) to stand or move due to skeletal and muscular pain and ataxia. In addition, swelling of costochondral

junctions and metaphyses and constipation were observed. The kittens assumed a standing position with a characteristic medial deviation of the paws. The kittens between the 5th to 8th weeks assumed sternal recumbency with the hind legs abducted and suffered from severe constipation as detected by palpation of abdomen. From the 5th week to the termination of the experiment after 9 weeks, the kittens were quiet and assumed sternal recumbency, lethargy and severe constipation *(Fig, 1-2).*

3.2. Biochemical changes:

Serum parathyroid hormone (PTH) and 1.25 (OH) 2 Vit.D3 of induced NSHPT group showed significant (P<0.05) increase from the 3rd week to the 9th week of induction. On the other hand, the 25 (OH) Vit.D3 showed significant (P<0.05) decrease from the 3rd to 9th week of induction compared with control group (Table 1). Serum enzymes CPK and ALP and also serum blood urea nitrogen (BUN) revealed significant (P<0.05) increase from the 3rd to 9th week of induction. On The contrary, serum creatinine, Total protein, albumin and globulin showed significant (P<0.05) decrease from the 3rd to 9th week of induction, but A/G ratio showed significant (P<0.05) increase from the 5th to 9th week of induction when compared with control healthy group *(Table 2).*

Serum calcium, magnesium, copper and zinc levels showed significant (P<0.05) decrease. However, serum phosphorus level showed significant (P<0.05) increase from the 3rd to 9th week of induction compared with control healthy group (Table 3).

3.3. Radiographic (X-Ray) changes:

The right lateral radiographs of whole body of cat with NSHPT revealed thoracolumbar kyphosis and lumbosacral lordosis. Showed severe reduction in radiopacity corresponding with severs osteopenia in bones of skull, softness and decreased opacity, fractures of bone of skull and marked generalized osteopenia and decreased bone opacity (Fig. 6). Demineralization of bones of radius and ulna were also demonstrated in addition to fracture of radius bone were also demonstrated showed in (Fig.7-8**)**

3.4. Postmortum (PM) changes:

PM examination reiterated softness of bone of skull in addition to fissure and fracture of bone of skull in cats with NSHPT**.** There were also fissures and fracture of bone of scapula in kittens with induced NSHPT (Fig. 3, 4 and 5).

Figure (1): Painful palapation of tarsals and metatarsals bones of posterior limb in four months cat with NSHPT .
posterior limb in four months cat with NSHPT

Figure (2): Incoordination in gait and lameness in the hind legs due to bone pain and bowing of the front legs in a four months cat with NSHPT.

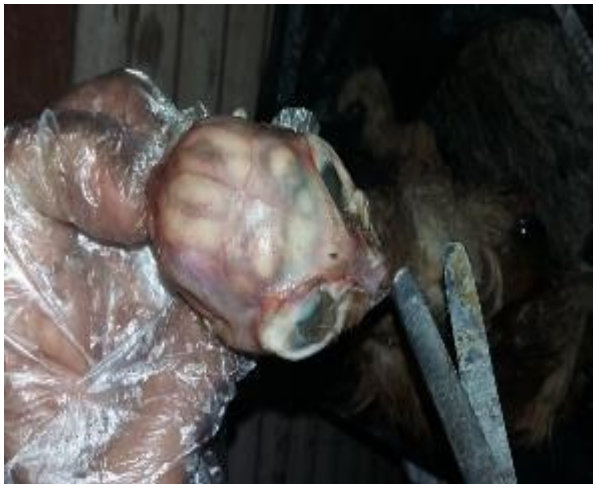

Figure (3): Severe thinning and softness of bones of skull in induced NSHPT in a four-month cat.

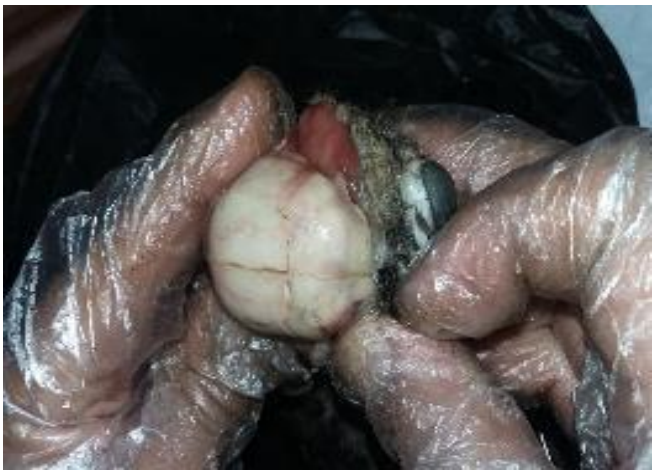

Figure (4): Fissured and fracture of the bone of skull in induced NSHPT in a four months cat.

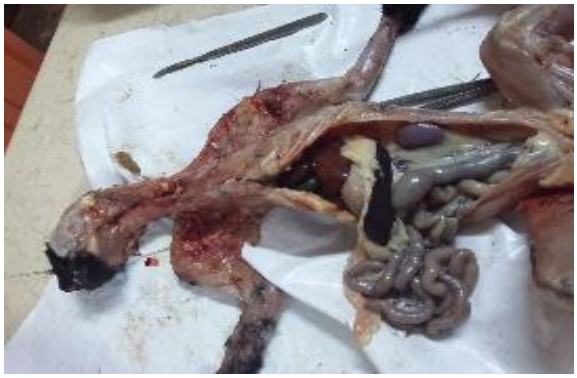

Figure (5): PM exam. Showed accumulation of hard food materials in small intestine and colon indicating severe constipation in induced NSHPT in four months cat.

Figure (6): Lateral radiographic examination of the whole skeleton bones of four month's cat with induced NSHPT showing generalized osteopenia with

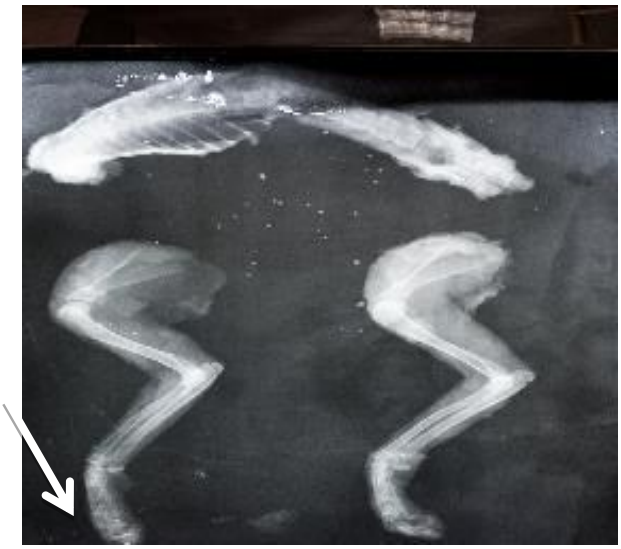

Figure (7): Radiographic examination of the bone of the fore limb in cat with NSHPT and control normal cat Evidence demineralization and fracture of radius and normal bone (Arrow).

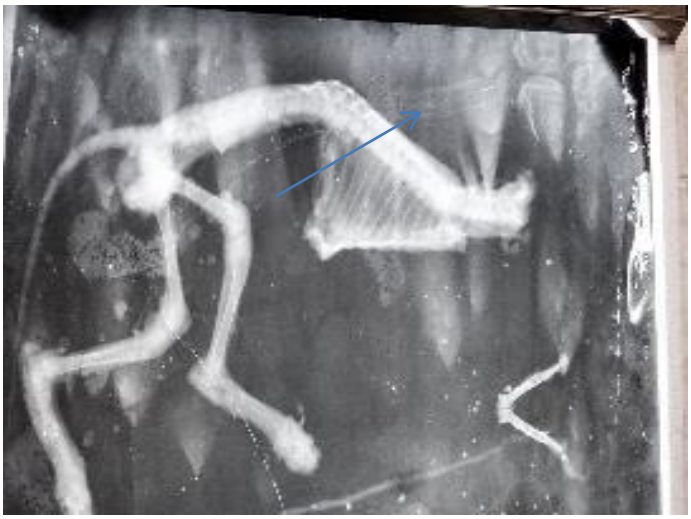

Figure (8): Lateral radiographic of the femurs, tibia, and fibula, cervical, thoracic, lumber, sacral and coccygeal vertebrae in four month's cats with induced NSHPT evidence decreased bone density.

Table (1): Serum hormones and vitamin analysis of control group and NSHPT group from the 3rd to 9th week of induction.

Parameters	Control group (n=10)	Induced group (n=10)			
		3rd week	5th week	7th week	9th week
PTH (Pg.\L)	13.57 [a] ±4.17	32.1 [b] ±2.22	47.45 [c] ±4	57.05 [d] ±5.66	59.6 [d] ±6.3
1.25-dihydroxy Vit.D3 (Pmol/L)	231.4[a] ±16.6	368.5[b] ±20.8	425.3 [c] ±24.9	438.3[c,d] ±26	453.7 [d] ±25.7
25-hydroxy Vit. D3 (Pmol/L	145.5 [a] ±6.55	99.75[b] ±5.43	65.2 [c] ±8.6	61 [c] ±9.5	66.35 [c] ±9.12
T4 µg /dL	0.928 [a] ±0.07	0.930 [a] ±0.09	0.939 [a] ±0.11	0.940[a] ±0.84	0.941 [a] ±0.12

Means with different superscript letters indicate significance difference among groups at P<0.05.

Table (2): Serum biochemical profile of serum enzymes and protein in control group and NSHPT group from the 3rd to 9th week of induction.

Parameter	Control group (n=10)	Induced group(n=10)			
		3rd week	5th week	7th week	9th week
CPK (U/L)	146.7 [a]±15.8	243.2 [b] ±22.8	297.6 [c] ±26.9	305.9[c,d] ±28.4	323.2 [d]±25.6
ALP (U/L)	32.4 [a]±6.64	70.6±8.1[b]	132.4±8.8[b]	139.6±14.1[c]	154.1±17.9[c]
BUN (mmol/L)	7.62 [a]±1.32	8.82 [b]±0.69	8.93 [c]±0.17	9.81 [c]±0.18	10.76 [c]±0.14
Creatinine(mmol/L)	61.55 [a]±8.67	46.01 [b]±3.79	40.15 [c]±4.6	37.9 [c]±4.2	36.25 [c]±4.43
TP (g/L)	91.75 [a]±8.1	78 [b]±2.8	73.65 [c]±3.59	72.8 [c]±3.26	71.85 [c]±3.51
Alb (g/L)	59.95 [a]±5.05	54.25 [b]±2.23	51.9 [b,c]±2.84	51.1 [c]±2.69	50.05 [c]±2.55
Glob (g/L)	31.8 [a]±5.28	23.75 [b]±1.65	21.75 [b]±1.43	21.7 [b]±1.56	21.8±1.18[c]
A/G ratio	1.885 [a]±0.29	2.284 [b]±0.15	2.386 [b]±0.14	2.355 [b] ± 0.1	2.296 [b]±0.12

Means with different superscript letters indicate significance difference among groups at P<0.05.

Table (3): Serum macro minerals and trace element analysis of control and nutritional -induced hyperparathyroidism group from the 3rd to 9th week of experiment.

Item	Control group (n=10)	Induced group (n=10)			
		3rd week	5th week	7th week	9th week
Ca (mmol/L)	2.203 [a] ±0.066	1.868 [b] ±0.067	1.412 [c] ±0.179	1.26 [d] ±0.08	1.225 [d] ±0.06
P (mmol/L)	1.468 [a] ±0.137	2.297 [b] ±0.303	2.81 [c] ±0.26	2.955[c,d]±0.189	3.07 [d] ±0.18
Mg (mEq/L)	2.14 [a] ±0.17	1.91 [b] ±0.21	1.48 [c] ±0.16	1.43 [c] ±0.16	1.40 [c] ±0.16
Cu (mmol/L)	7.463 [a] ±0.216	7.167 [b] ±0.162	5.9 [c] ±0.29	5.697[c,d]±0.339	5.64 [d] ±0.31
Zn (mmol/L)	1.037 [a] ±0.13	0.772 [b] ±0.12	0.584 [c] ±0.087	0.56 [c] ±0.09	0.529 [c] 0.082

Means with different superscript letters indicate significance difference among groups at P<0.05

4. DISCUSSION:

The common clinical signs of diseased cats with NSHPT included anorexia, depression, lethargy, vomiting and constipation. Cats developed seizures due to low calcium in meals, reluctance to move due joint pain secondary to nerve root compression. There was also incoordination in gait and lameness in the back legs while the front legs were often bowed and their thin bones and easily fractured due to mobilization of calcium from bone under effect of PTH. These signs resembled those observed by Moarrabi *et al.* (2008), Dimopoulou *et al.* (2010), Pedram *et al.* (2014) and Parker *et al.* (2015).

The biochemical examination showed a gradual increase in serum parathyroid hormone (PTH) concentration from the 3rd week of induction till the end of experiment in comparison with control group. These results agreed with those reported by Tomsa *et al*. (1999) and Nagata and Yuki (2013). This result could be attributed to the low calcium and high phosphorus content of the beef heart meat that lead to hypocalcaemia with subsequent hypertrophy of the parathyroid glands, thus increasing secretion of PTH. The serum 1, 25-dihydroxy vitamin D3 level was significantly increased from the 3rd week of induction to the end of experiment. On contrast, serum 25-hydroxy vitamin D3 level was significantly decreased. These results coincided with Tomsa *et al*. (1999) and Parker *et al.* (2015). The elevated PTH in response to decreased blood calcium level stimulates the 1-α-hydroxylase, which is a renal enzyme that increased renal hydroxylation of 25 (OH) induction vitamins D3 to 1, 25- activity (Parker *et al.,* 2015). The dihydroxy vitamin D3 (calcitrol) enhances the absorption of Ca and P from intestine, but in bone, promotes the release of Ca and P into extracellular fluid through stimulating osteocyte activity and suppressing osteoplastic.

Serum CPK showed a significant increase from the 3rd week of induction to the end of experiment in comparison with control group. This result agreed with Nagata and Yuki (2013) that stated that elevated CPK could be attributed to the effect of stress of nutritional secondary hyperparathyroidism and due to muscles damage and this was very common in ill cats.

Serum ALP level showed significant increase from the 3rd week of induction to the end of experiment. This result coincided with Tomsa *et al.* (1999), Moarrabi *et al*. (2008) and Parker *et al* (2015) . The high ALP level could be attributed to the elevated bone isoenzyme associated with growth and may be attributed to bone problems such as weakening of bones related to deficiency of calcium, inability of the body to break down vitamin D and an overactive parathyroid gland. Serum BUN was marked high level but also marked lower in creatinine in the second group of nutritional-induced hyperparathyroidism cats that showed significant ($p \leq 0.05$) increased in level of BUN and decreased in level of Creatinine and this due to disturbance in kidney and liver function and effect of PTH and increased P and decreased Ca levels that agreed with Den-Hertog et al (1997) and Nagata and Yuki (2013).

Serum biochemical profiles in nutritional-induced hyperparathyroidism cats showed significant ($p \leq 0.05$) decreased in serum total protein, albumin and globulin level from the 3rd week of induction to the end of experiment in comparison with control group. These findings are in agreement with Nagata and Yuki (2013) and this result could be attributed to the effect of malnutrition in some cases of

NSHPT, and depression of immunity and suppression of plasma protein synthesis secondary to reduction of the metabolism process.

Serum calcium level showed gradual decrease while serum inorganic phosphorus revealed gradual increase from the 3nd week of induction till the end of experiment. These results matched those reported by Moarrabi *et al.* (2008), Nagata and Yuki (2013), and Pedram *et al* (2014) who reported that serum calcium levels were consistently low in the cats with NSHPT, that might be attributed to the low Ca content, high P of all meat diets (phosphorus to calcium ratio 25-1), rapid bone metabolism .The serum phosphorus levels were consistently high and this could be attributed to rapid bone metabolism and high P content of meat diet. Serum Mg, Cu and Zn levels showed significant ($p \leq 0.05$) decrease from the 3nd week of induction until the end of experiment compared to control group. The decrease in serum Mg concentrations in NSHPT could be attributed to mobilization of Mg from bone and increased urinary excretion as explained by (Robert and Rude 1998). Cu deficiency was directly due to severe osteoporotic lesions, mild dietary Cu deficiency and PTH increased urinary excretion of Cu in cats as demonstrated by (Strain 2004) and Pedram *et al* (2014). Moreover, the significant decrease in serum Cu and Zn concentration may be attributed to urinary excretion of copper and zinc is greater in case of hyperparathyroidism than normal.

Radiographic examination showed marked generalized decreased bone opacity, osteopenia, thin cortices and fractures of long bone in cats with NSHPT compared with control. The bone change could be attributed to mobilization of Ca from the bone under the effect of PTH due to NSHPT, as explained by Graham et al., (2010).

The constipation in cats with NSHPT could be attributed to lack normal smooth muscle function of colon under effect of low Ca ions; this result is agreement with Chattopadhyay *et al*., (1996).

5. CONCLUSION:

NSHPT in cats can be readily induced by feeding low Ca-high P diet such as heart beef meat. The clinical signs appeared on the 4th week post induction. In addition to the clinical alterations, the NSHPT was associated with biochemical changes from the 4th week post induction (i.e.: one week before the clinical signs). The radiographic exam. Confirmed the bone changes that were reflected on the clinical signs. Therefore, it is recommended to provide cats with a balanced diet, especially during the first weeks of life which considered as a key factor for prevention of NSHPT in cats.

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