Evaluation of Four Treatment Protocols on Experimentally Induced Nutritional Secondary Hyperparathyroidism in Kittens

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A B S T R A C T

The aim of this study was to compare the effect of different treatment in cat models of induced nutritional secondary hyperparathyroidism (NSHPT) by using chicken's eggs hell compared to other conventional treatments. The NSHPT was induced experimentally in 20 rapidly growing kittens by feeding a diet consisted of only beef heart meat (low Ca-high P) for 9weeks. The clinical signs of NSHPT in kittens included disturbance in locomotion manifested by reluctance to move, posterior lameness, and uncoordinated gait. The diseased kittens were divided into four equal groups (n=5), each group was treated with a different protocol. Treatment of affected kittens was achieved by eggshell (G4) that was compared to three treatment protocols including combined Ca-vitamin D injection. (G1), oral Ca alone (G2) and oral Ca, Mg, and Zn (G3). The clinical symptoms and biochemical analysis were monitored at day 1, 3, 5 and 7 after treatment. The PTH and 1.25 (OH) 2 Vit.D3were gradually reduced starting from the 1st day post treatment in eggshell treated group compared to other treatment groups. The eggshell treatment induced changes in clinical signs and biochemical parameters that were comparable to the other three groups. Based on these results, it was suggested that feeding egg shell solution could be used as an economic source of Caasan alternative treatment for NSHPT in cats.

Keywords: Calcium, Egg shell, Kittens, NSHPT, Vitamin D.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 182-194, 2018)**

1. INTRODUCTION:

Nutritional secondary hyperparathyroidism (NSHPT) is caused by chronic hypocalcaemia due to persistent deficiency of calcium in diet (Williams 2007). NSHPT is a generalized metabolic bone disease characterized by osteopenia and caused by inadequate calcium in the diet, excessive phosphorus, or a combination of both (Nap and Hazewinkel,1994) and (Moarrabi *et al.*,2008). The most common cause of NSHPT is dietary insufficiency of calcium or vitamin D and excess phosphorus (Phillips *et al*., 2011). The clinical manifestations of NSHPT in kittens are anorexia, weight loss, depression, hyperesthesia, constipation, joint pain and lameness. In advanced cases the neck becomes rigid due to ankylosis of the cervical vertebrae, lameness in the forelimb and pain owing to nerve root compression. Kittens with NSHPT were stunted and have limb deformity (Scott and Meloughlin,2007). The short-term parenteral calcium gluconate injection, a balanced diet and cage rest were the treatment of cats with NSHPT (Tomsa *et*

al (1999) and Aldavood *et al.,* (2009). The conventional treatment consisted of intravenous administration of calcium in the form of 10% calcium gluconate (5-15 mg / kg) given slowly over 10-30 minutes that give the effect immediately beside that oral supplementation with vitamin D and calcium is needed (Lappin 2001). Calcium carbonate represents 95% of chicken eggshell and 3.5% is organic mixtures. The chicken eggshell which is rich in calcium carbonate was used in treatment of calcium deficiency in human and animals (Daengprok *et al* 2003). The weight of one eggshell is 6 grams that contained 2200 mg of calcium (Vlad, 2009). The treatment of hypocalcaemia by using of 10% calcium gluconate could be disadvantageous because it may be accompanied by bradycardia (Scott *et al* 2014). Therefore, the treatment of NSHPT by oral Ca carbonate and vit D was proposed (Asi*et al* 2014).

In this study, we used the eggshell as a natural therapy for cats with experimentally induced NSHPT and compare the result with other treatment protocols including combined Ca and Vit. D injection, oral Ca alone and oral Ca, Mg and zinc.

2. MATERIALS AND METHODS:

2.1. Animals and experimental design

The present study was carried out on 20 apparently healthy male kittens aged from 2 to 3months, and their body weight ranged between 0.45-0.85 kg. All the 20 male kittens were experimentally induced with NSHPT by feeding a diet of only beef heart. Iodine was added to maintain healthy thyroids and tap water was supplied *ad-libitum* according to the method described by Rowland et al (1968). This diet induced the NSHPT after feeding for 9 weeks. After complete induction of NSHPT in young male cats that ended on the 9th week of induction that considered the zero days (0-day) of the beginning of treatment trials, the 20 kittens were randomized into 4 groups, each group was treated with a different treatment protocol. The first group (G1) was treated by calcium gluconate and Vitamin D injection (calcium gluconate injection, (5-15 mg / kg body weight was given slowly I/V over 10-30 minutes as a single dose (Cal bor mag. Inj. ADWIA PHARM. CO. EGYPT. Dosage and administration: Cats: 5 ml. I/V or S/C inj. and Vitamin D3 inj. MEMPHIS COM. For pharm &chem. Ind. EGYPT. Single dose 5000- 10000 I.U I/M. may be repeated after 10-15 days for 4 times, or ½ Amp every 6 months. The second group (G2) was treated by Kalsi-um 2000 Tablets as recommended by manufacturer (dose: 1/2- 1 Tablet/cat/day for5-7daysPO) Mark & Chappell. UK. The third group (G3) was treated by Osteocare tablets as recommended by manufacturer (Vitabiotics, Egypt M.O.H. Reg. No.:819/2009). Each tablet contains calcium carbonate 1000mg (400 mg),magnesium 359.79 mg(150 mg), zinc sulphate 22mg (5mg) and cholecalciferol 0.2mg (500,000 I U/g) (100 IU). The fourth group (G4) was treated by eggshell solution prepared from eggshell powder as described later. The clinical and biochemical results were recorded from the 0-day, 1st day, 3rd day, 5th day and ended on the 7th day of treatment.

2.2. Preparing of eggshell powder and solution

2.2.1. Eggshell powder

Eggshell was dried in the sun or oven on the sheet for baking for 5 minutes to dry then ground in the grinder to get the powder of eggshell according to method of Brun*et al*. (2013).

2.2.2. Egg shell solution

One teaspoon of eggshell powder (equivalent to 2000-3000 mg Ca) was dissolved in 20 ml of milk then boiled and allowed to cool down. The eggshell solution was given orally once daily for 7 days. (The Weight of one eggshell equal six grams that contained 2200mg of calcium.).The dose used was 1 Eggshell powder /orally /day for 7 days (Biova, 2009).

2.3. Clinical examination

The Kittens were subjected to clinical examination and all clinical signs were recorded before and after treatment as previously described (Kelly, 1984).

2.4.Biochemical analysis

2.4.1.Blood samples

Blood samples were collected from cephalic or jagular vein according to Kirk and Bistner (1985). The blood samples (5ml) were divided into two portions, one with anticoagulant (potassium salt of EDTA) for hematological examinations and the other portion without anticoagulant for obtaining a clear non hemolyzed serum by centrifugation of the blood sample at 3000 r.p.m. for 5 minutes. The clear sera were aspirated carefully by automatic pipette and transferred into clean dry labeled Eppendorf tubes, and stored at −20°Ctill examination. Only clear non hemolyzed serum was used. Special chemical kits (Produced by Bio-analytic Company, Egypt) were used for determination of serum parathyroid hormone (PTH), 25-hydroxyvitamin D3, 1, 25-di hydroxyvitamin D3, BUN, creatinine, creatinine phosphokinase (CPK), alkaline phosphatase (ALP), calcium (Ca), phosphorus, copper (Cu), zinc (Zn) ,magnesium (Mg) , total protein (TP) ,albumin (Alb) and globulin (glob) were determined at 0-day, 1st day, 3rd day, 5th day and 7th day of treatment according to method described by Tomsa et al (1999).

2.5. Statistical analysis

Statistical analysis of results was carried out using two ways analysis of variance (ANOVA) with Duncan's post hoc test as previously described (Bailey,2008) using sigma stat software to test both treatment and times effect. The results were demonstrated as means ± SE. The results were considered statistically significant when $p<0.05$.

3. RESULTS:

3.1. The clinical signs:

Cats treated with injectable Ca and vit. D (G1) showed improvement of movement, decreased acute seizures and the following day developed generalized muscles, decreased joint pain andlameness. Treated cats developed normal defection and ability to stand and move. While cats of G2 showed gradual improvement of movement, and ability to stand and move after one to four days. Defection improved on the 3 days post of treatment but joint pain and lameness improved on the 7 day of treatment. Cats in group BP3 showed gradual improvement of movement, and ability to stand and move after one to three days. Defection improved after the4th days but joint pain and lameness improved from day 7 of treatment. Cats in G4 showed gradual improvement of movement, and ability to stand and move after three to four days. Defection improved 3 days aftertreatment but joint pain and lameness improved from the 6th day of treatment *(Fig,1, 2).*

3.2. Serum biochemical changes:

The obtained data in table 1 showed symptoms of decrease in serum parathyroid hormone (PTH) and 1,25 (OH)2 Vit.D3 beginning from the 1st day of treatment in all groups of treatment with only significant (P<0.05) decrease in the first group of treatment (G1) than the other three groups. There were non-significant changes between groups on the other periods of treatment. On the other hand, serum 25 (OH) Vit.D3 revealed a significant (P<0.05) increase from the 1st day of treatment with no significant changes observed among groups.

The data presented in table 2 demonstrated that, serum CPK activity revealed significant (P<0.05) decrease from the 1st day of treatment in all groups of treatment with only significant (P<0.05) decrease in the first group than the other groups of treatment. Serum ALP activity revealed a significant (P<0.05) decrease from the 1st day of treatment in all treatment groups with no significant changes observed between them all over the period of treatment.

The data demonstrated in table 3 revealed that serum total protein confected significantly (P<0.05) increased from the 3rd day of treatment in all treatment groups with no significant changes among groups all over the period of treatment. On the other hand, serum albumin level result was significantly (P<0.05) increased from the 3rd day of treatment in the first group but from the 1st day of treatment in the other groups of treatment. Serum globulin was showed a significant (P<0.05) increase in the first and fourth groups of treatment (from the 1st to 7th day of treatment compared with the 0-day, while there was a significant (P<0.05) increase in the second and third groups of treatment on the 5th and 7th day of treatment compared with the 0-day. The A/G ratio revealed a significant (P<0.05) decrease only in the first group of treatment on the 1st day of treatment compared with the third group of treatment

The data presented in table 4 showed that serum calcium level showed a significant (P<0.05) increased from the 1st day of treatment compared with 0-day in all groups of treatment. However serum phosphorus level was significantly (P<0.05) decreased from the 3rd day of treatment in the first, second and third groups of treatment but from the 1st day of treatment in the fourth group of treatment. Serum magnesium revealed a significant (P<0.05) increase from the 3rd day of treatment in all treatment groups with non-significant changes between groups all over the period of treatment. The serum copper level revealed a significant (P<0.05) increase from the 3rd day of treatment in all groups of treatment with only significant (P<0.05) on the 3rd day of treatment in G1 and G1 and G4 compared with G2 and G3. Serum zinc level was revealed significantly (P<0.05) increased from the 3rd day of treatment in G2, G3, and G4 but from the 5th day of treatment in the G1 compared to the 0-day with non-significant changes between groups of treatment all over the period of treatment.

Figure (1): Incoordination gait and lamenessthe hind legs due to bone abnormalities in a four months cat with NSHPT

Figure (2) : Reluctance to move due to bone abnormalities in a four months cat with NSHPT

Figure (3) : Normal position of cat with NSHPT after being treated by egg shell protocol.

Table (1): Serum hormones and vitamin analysis before and after treatment

Parameters	Period	G1 (n=5)	G2 (n=5)	G3 (n=5)	G4 (n=5)
PTH Pg/L	0 day	54.75^{a,1}±4.25	56.75^{a,1}±4.25	59.75^{a,1} ±2.25	60.25^{a,1}±6.75
	1st day	39.5^{a,2}±2.5	48.75^{b,2}±4.75	48.5^{b,2}±2.5	48.75^{b,2}±5.75
	3rd day	33.25^{a,3}±2.25	34.75^{a,3}±6.25	34.25^{a,3}±3.25	34.5^{a,3}±4.5
	5th day	22.25^{a,4}±1.88	24.75^{a,4}±3.75	24.5^{a,4}±2.25	24.5^{a,4}±3.5
	7th day	17.25^{a,5}±1.75	18.5^{a,5}±2.5	17.25^{a,5}±2.75	17.5^{a,5}±2.25
1.25(OH)2Vit D3 (Pmol/L)	0 day	461^{a,1}±26	478.3^{a,1}±26.8	477.8^{a,1}±32.8	474.8^{a,1}±28.3
	1st day	385^{a,2}±10	425.5^{b,2}±14.5	425^{b,2}±22.5	423^{b,2}±13.5
	3rd day	335^{a,3}±4	352.5^{a,3}±5	354.3^{a,3}±4.63	350.75^{a,3}±5.38
	5th day	279^{a,4}±6.5	280.5^{a,4}±7	280^{a,4}±4	277.5^{a,4}±5
	7th day	252.5^{a,5}±5.75	254^{a,5}±7	254.3^{a,5}±5.75	252.5^{a,5}±5
25(OH)Vit D3 (Pmol/L)	0 day	82.5^{a,1}±12	78.25^{a,1}±11.38	72.25^{a,1}±9.25	76.75^{a,1}±4.63
	1st day	104^{a,2}±3	90.75^{b,2}±5.38	91^{b,2}±4.5	90.25^{b,2}±5.25
	3rd day	123^{a,3}±3.5	117.75^{a,3}±3.25	118^{a,3}±4	118.5^{a,3}±3.5
	5th day	130.5^{a,3,4}±3.75	128^{a,3}±6.5	129.3^{a,3,4}±3.8	130^{a,3,4}±4.5
	7th day	142^{a,5}±5	141.3^{a,5}±3.75	141.5^{a,5}±4	140.5^{a,5}±4.25

Superscript letters: Mean significance difference between groups on P<0.05.

Superscript numbers: Mean significance difference among times of treatment in the same group on P<0.05

G1:Treated by Calcium Gluconate 10% +Vit D inj.

G2:Treated by Kalsi-um Tablets

G3:treated by Osteocare Tablets

G4: treated by Eggshell powder

Table (2): *Serum CPK, ALP, BUN, and Creatinine concentration* before and after treatment of kittens with NSHPT.

Parameters	Period	G1 (n=5)	G2 (n=5)	G3 (n=5)	G4 (n=5)
CPK (U/L)	0 day	$246.8^{a,1}\pm9.13$	$245.8^{a,1}\pm10.13$	$246^{a,1}\pm12$	$246.5^{a,1}\pm11.8$
	1st day	$212^{a,2}\pm4.5$	$228.5^{b,2}\pm6.5$	$227^{b,2}\pm6$	$225.3^{b,2}\pm6.75$
	3rd day	$202^{a,2,3}\pm4.5$	$214^{a,3}\pm3.5$	$212.5^{a,3}\pm4$	$211.3^{a,3}\pm6.25$
	5th day	$195^{a,3}\pm3.5$	$204.5^{a,3}\pm3.25$	$202^{a,3}\pm3.5$	$200.8^{a,3}\pm4.63$
	7th day	$179.8^{a,4}\pm4.88$	$180.3^{a,4}\pm3.75$	$182^{a,4}\pm3.5$	$182.5^{a,4}\pm5$
ALP (U/L)	0 day	$158.8^{a,1}\pm16.3$	$160.3^{a,1}\pm27.3$	$161.8^{a,1}\pm23.8$	$161.5^{a,1}\pm25.5$
	1st day	$107.3^{a,2}\pm6.75$	$119^{a,2}\pm6.55$	$120^{a,2}\pm6.5$	$120.5^{a,2}\pm10.5$
	3rd day	$83.5^{a,3}\pm5.75$	$89.5^{a,3}\pm7.25$	$89^{a,3}\pm6.5$	$89.5^{a,3}\pm5.75$
	5th day	$52.5^{a,4}\pm6.25$	$55.5^{a,4}\pm7.75$	$54^{a,4}\pm7$	$53.75^{a,4}\pm6.75$
	7th day	$41^{a,4}\pm6$	$42^{a,4}\pm6$	$41.5^{a,4}\pm5.5$	$41.5^{a,4}\pm5.75$
BUN (mmol/L)	0 day	$7.98^{a,1}\pm0.18$	$8.99^{a,1}\pm0.29$	$9.94^{a,1}\pm0.29$	$10.5^{a,1}\pm0.29$
	1st day	$7.18^{a,1,2}\pm0.15$	$8.11^{a,1}\pm0.23$	$9.06^{a,1}\pm0.23$	$9.38^{a,1,2}\pm0.39$
	3rd day	$6.45^{a,2,3}\pm0.12$	$6.36^{a,1,2}\pm0.23$	$7.31^{a,1,2}\pm0.23$	$7.56^{a,2,3}\pm0.29$
	5th day	$5.58^{a,2,3}\pm0.06$	$5.68^{a,2,3}\pm0.08$	$5.63^{a,2,3}\pm0.08$	$5.8^{a,3,4}\pm0.03$
	7th day	$5.53^{a,3}\pm0.17$	$5.51^{a,3}\pm0.11$	$5.56^{a,3}\pm0.11$	$5.59^{a,4}\pm0.06$
Creatinine (mmol/L)	0 day	$36.25^{a,1}\pm2.25$	$36^{a,1}\pm2.5$	$37.5^{a,1}\pm2.5$	$37.25^{a,1}\pm2.75$
	1st day	$45.5^{a,2}\pm2.75$	$45.5^{a,2}\pm4$	$45.75^{a,2}\pm5.25$	$45^{a,2}\pm4$
	3rd day	$50.5^{a,2,3}\pm3.5$	$53.3^{a,2,3}\pm3.63$	$53.75^{a,3}\pm4.38$	$52^{a,2,3}\pm4.5$
	5th day	$57.25^{a,3,4}\pm4.25$	$58.75^{a,3,4}\pm2.13$	$58.75^{a,3,4}\pm4.38$	$57.75^{a,3,4}\pm2.88$
	7th day	$62^{a,4}\pm4$	$63.25^{a,4}\pm2.75$	$62.5^{a,4}\pm4.5$	$61^{a,4}\pm3.5$

Superscript letters: Mean significance difference between groups on $P<0.05$.

Superscript numbers: Mean significance difference among times of treatment in the same group on $P<0.05$

G1:Treated by Calcium Gluconate 10% +Vit D inj. – G2:Treated by Kalsi-um Tablets

G3:treated by Osteocare Tablets – G4: treated by Eggshell powder.

Table (3): Serum total protein and its fractions before and after treatment of kittens with NSHPT.

5	Period	G1 (n=5)	G2 (n=5)	G3 (n=5)	G4 (n=5)
Total Protein (g/l)	0 day	72.5 ^{a,1}±7.25	74.5 ^{a,1} ±6.5	73.5 ^{a,1} ±6.75	75.75 ^{a,1} ±6.75
	1st day	78 ^{a,1,2}±2.5	82.25^{a,1,2}±2.63	81.25^{a,1,2}±2.63	83.5 ^{a,1,2}±2.25
	3rd day	82.25^{a,2,3}±2.25	85 ^{a,2,3}±2	84 ^{a,2,3}±2	86.75^{a,2,3}±2.25
	5th day	86 ^{a,2,3}±2.5	90.5 ^{a,2,3}±3	89.5 ^{a,2,3}±3	90.25^{a,2,3}±2.75
	7th day	88.5 ^{a,3}±2	92.75 ^{a,3}±3.75	91.75 ^{a,3}±3.75	92.75^{a,3}±3.88
Albumin (g/l)	0 day	50.75 ^{a,1}±2.25	51.75 ^{a,1}±1.25	51.5 ^{a,1}±1.5	53 ^{a,1}±1.5
	1st day	51.75 ^{a,1}±2.25	57.75 ^{b,2}±1.88	57.5 ^{b,2}±1.75	58.5 ^{b,2}±1.75
	3rd day	56.75 ^{a,2}±1.75	62 ^{b,2,3}±2	60.25^{a,b,2,3}±1.75	61.5 ^{b,2,3}±2.5
	5th day	60.5 ^{a,2}±2.25	63.75^{a,3,4}±2.75	63.5 ^{a,3,4}±2	64 ^{a,3,4}±2
	7th day	61 ^{a,2}±2	66.75^{b,4}±2.13	66.5 ^{b,4}±2.25	68.25 ^{b,4}±2.25
Globulin (g/l)	0 day	21.75 ^{a,1}±0.75	22.75^{a,1}±0.75	22 ^{a,1}±1.5	22.75 ^{a,1}±0.75
	1st day	26.25 ^{a,2}±1.25	24.5 ^{a,1,2,3}±1	23.75 ^{a,1,2}±1.25	25 ^{a,1,2}±1.5
	3rd day	24.5 ^{a,2}±1.5	23 ^{a,1,2}±1	23.75 ^{a,1,2}±1.75	25.25^{a,1,2}±1.75
	5th day	25.5 ^{a,2}±1.5	26.75^{a,3}±1.25	26 ^{a,2}±1.5	26.25 ^{a,2}±1.25
	7th day	27.5 ^{a,2}±1.5	26 ^{a,2,3}±2	25.25 ^{a,2}±1.88	24.5 ^{a,2}±1.75
A/G ratio (%)	0 day	2.34 ^{a,1}±0.11	2.28 ^{a,1}±0.11	2.34 ^{a,1}±0.16	2.32 ^{a,1}±0.12
	1st day	1.99 ^{a,1}±0.38	2.36 ^{a,b,1}±0.12	2.5 ^{b,1}±0.15	2.34 ^{a,b,1}±0.16
	3rd day	2.32 ^{a,1}±0.12	2.7 ^{a,1}±0.32	2.54 ^{a,1}±0.24	2.44 ^{a,1}±0.22
	5th day	2.37 ^{a,1}±0.18	2.39 ^{a,1}±0.19	2.44 ^{a,1}±0.19	2.44 ^{a,1}±0.18
	7th day	2.22 ^{a,1}±0.31	2.57 ^{b,1}±0.2	2.66 ^{b,1}±0.22	2.79 ^{b,1}±0.3

Superscript letters: Mean significance difference between groups on P<0.05.

Superscript numbers: Mean significance difference among times of treatment in the same group on P<0.05

G1:Treated by Calcium Gluconate 10% +Vit D inj. – G2:Treated by Kalsi-um Tablets

G3:treated by Osteocare Tablets – G4: treated by Eggshell powder

Table (4): Changes of serum Ca, P, Mg, Cu, and Zn concentrations before and after treatment of kittens with NSHPT.

Parameters	Period	G1 (n=5)	G2 (n=5)	G3 (n=5)	G4 (n=5)
Ca (mmol/L)	0 day	1.32 ^{a,1}±0.08	1.29 ^{a,1}±0.07	1.28 ^{a,1}±0.07	1.27 ^{a,1}±0.07
	1st day	2.05 ^{a,2}±0.07	1.65 ^{b,2}±0.05	1.64 ^{b,2}±0.05	1.75 ^{b,2}±0.05
	3rd day	2.14 ^{a,2,3} ±0.09	1.91 ^{b,3}±0.06	1.9 ^{b,3}±0.06	1.98 ^{b,3}±0.01
	5th day	2.19 ^{a,3}±0.04	2.02 ^{b,3}±0.04	2.02 ^{b,3}±0.05	2.16 ^{a,b,4}±0.12
	7th day	2.35 ^{a,4}±0.03	2.33 ^{a,4}±0.13	2.33 ^{a,4}±0.08	2.33 ^{a,5}±0.08
P (mmol/L)	0 day	3.06 ^{a,1}±0.08	3.13 ^{a,1}±0.07	3.14 ^{a,1}±0.07	3.36 ^{a,1}±0.12
	1st day	2.85 ^{a,1,2}±0.2	2.81 ^{a,1}±0.23	2.81 ^{a,1}±0.23	2.63 ^{a,2}±0.26
	3rd day	2.6 ^{a,2,3}±0.16	2.44 ^{a,2}±0.29	2.46 ^{a,2}±0.29	2.36 ^{a,2,3}±0.22
	5th day	2.36 ^{a,3,4} ±0.13	2.27 ^{a,2,3}±0.2	2.22 ^{a,2,3}±0.15	2.21 ^{a,3,4}±0.2
	7th day	2.04 ^{a,4}±0.05	2.03 ^{a,3}±0.03	2.01 ^{a,3}±0.02	1.98 ^{a,4}±0.05
Mg (mEq/L)	0 day	1.42 ^{a,1}±0.1	1.62 ^{a,1}±0.14	1.61 ^{a,1}±0.13	1.63 ^{a,1}±0.14
	1st day	1.62 ^{a,1,2}±0.07	1.77 ^{a,b,1,2}±0.11	1.90 ^{a,b,1,2}±0.1	1.91 ^{b,2}±0.06
	3rd day	1.79 ^{a,2,3}±0.04	1.9 ^{a,2}±0.02	1.97 ^{a,2,3}±0.04	1.98 ^{a,2}±0.005
	5th day	1.91 ^{a,3}±0.01	1.955 ^{a,2}±0.015	2.01 ^{a,2,3}±0.03	2.02 ^{a,2,3}±0.04
	7th day	1.96 ^{a,3}±0.03	1.8 ^{a,1,2}±0.33	2.21 ^{a,b,3}±0.07	2.21 ^{b,3}±0.09
Cu (mmol/L)	0 day	5.56 ^{a,1}±0.34	5.55 ^{a,1}±0.3	5.45 ^{a,1}±0.3	5.82 ^{a,1}±0.19
	1st day	6.05 ^{a,1}±0.23	5.99 ^{a,1,2}±0.26	5.89 ^{a,1,2}±0.26	6.11 ^{a,1}±0.19
	3rd day	6.78 ^{a,b,2}±0.21	6.39 ^{a,2}±0.49	6.29 ^{a,2}±0.49	6.89 ^{b,2}±0.06
	5th day	7.3 ^{a,3}±0.1	7.16 ^{a,3}±0.21	7.06 ^{a,3}±0.21	7.41 ^{a,3}±0.17
	7th day	7.56 ^{a,3}±0.14	7.56 ^{a,3}±0.06	7.46 ^{a,3}±0.06	7.71 ^{a,3}±0.11
Zn (mmol/L)	0 day	0.59 ^{a,1}±0.11	0.55 ^{a,1}±0.05	0.54 ^{a,1}±0.045	0.61 ^{a,1}±0.03
	1st day	0.65 ^{a,1}±0.13	0.62 ^{a,1,2}±0.05	0.66 ^{a,1,2}±0.05	0.67 ^{a,1,2}±0.02
	3rd day	0.72 ^{a,1,2}±0.13	0.71 ^{a,2,3}±0.06	0.73 ^{a,2,3}±0.06	0.73 ^{a,2,3}±0.045
	5th day	0.85 ^{a,2,3}±0.07	0.8 ^{a,3}±0.01	0.82 ^{a,3}±0.01	0.81 ^{a,3,4}±0.02
	7th day	0.9 ^{a,3}±0.1	0.85 ^{a,3}±0.02	0.88 ^{a,3}±0.02	0.87 ^{a,4}±0.015

Superscript letters: Mean significance difference between groups on P<0.05.

Superscript numbers: Mean significance difference among times of treatment in the same group on P<0.05

G1:Treated by Calcium Gluconate 10% +Vit D inj. – G2:Treated by Kalsi-um Tablets

G3:treated by Osteocare Tablets – G4: treated by Eggshell powder

4. DISCUSSION:

The major clinical signs of cats with NSHPT were anorexia, lameness, and irregularity of the vertebral column and long bones. Most of these cats referred with pathological fractures and a reluctance to move, posterior lameness, and in coordinated gait. Affected kittens developed constipation,

tremor, muscle twitching, seizures and ataxic gait. Pain was observed due to nerve root compression related to NSHPT. Treatment was limited to short-term parenteral calcium gluconate injections, Catablets orally, to induce quick clinical recovery of cats with NSHPT. The marked improvement in clinical signs about 70-75% after the first dose of treatment with the first protocol of treatment (Ca and Vit D inj.). This result was comparable with the result of treatment of NSHPT recorded by Tomsa et al. (1999)who observed disappearance of clinical signs in cats 1-3 days post injection. On the other hand, treatment with second, third and fourth protocols improve in clinical signs after the third dose of treatment.

Biochemically, serum parathyroid hormone (PTH) and 1.25 (OH) Vit.D3 were significantly decreased from the 1st day of treatment in all groups of treatment with only significant decrease in the first group of treatment (BP1) than the other three groups with non-significant changes between groups on the other periods of treatment. These results are nearly similar with Tomsa et al., (1999), Nagata and Yuki (2013) and Parker et al., (2015).The decrease of PTH level after treatment could be attributed to the rapid response of PTH secretions to changes in serum calcium. Once sufficient calcium source if given, serum ionized calcium normalized and PTH secretion drop rapidly and this result agreed with (Barber *et al.*,1993) who had similar results. In this study, the eggshell produced comparable results in terms of the level of PTH compared to the other conventional treatment.

Serum 25 (OH) Vit.D3 revealed significant increase from the 1st day of treatment in all groups. This result agreed with Tomsa et al.(1999) and Parker et al.(2015).This result may be attributed to increased blood level of calcium related Ca supplementation, increased Vit.D3 content of the diets for these cats and decreased hydroxylation of 25(OH) Vitamin D3 to 1,25(OH)2Vitamin.

Serum CPK activity was significantly decreased from the 1st day of treatment in all groups of treatment with non-significant changes between groups. This result agreed with Nagata and Yuki (2013). The CPK is an enzymatic marker for muscular damage secondary to NHPT and its reduction following treatment could be attributed to decreased skeletal muscles damages (Aroch *et al* (2010) , Watanabe and Duque (2011) and Nagata and Yuki ., 2013).

However, serum ALP activity revealed a significant gradual decrease from the 1st day of treatment in all groups of treatment with non-significant changes between them all over the period of treatment. The activity of serum alkaline phosphatase (ALP) was higher in affected cats with NSHPT, under the effect of high levels of PTH, P and low calcium level ,after treatment , the level of PTH ,P and calcium were corrected and within normal level states, so the ALP *decreased Moarrabi et al (2008*).

Serum BUN revealed a significant gradual decreased from the 1stday of treatment in all groups of treatment. On the other hand, the serum creatinine also gradually increased near the normal range from the 1st day of treatment in all groups of treatment *(Table, 2)*. This result agreed with Samra (2012) and Nagata and Yuki (2013). This result could be attributed to the improvement of liver and kidney function, the PTH and P, Ca ratio due to offering balanced prepared cats food and medicated drugs in addition to decrease of bone pain and stress so cats can easily move and eat much.

Serum total protein gradually increased began the 3rd day of treatment in all groups of

treatment with non-significant changes between them all over the period of treatment. On the other hand, serum albumin gradually increased from the 3rd day of treatment in the first group of treatment (G1) but from the 1st day of treatment in the other groups of treatment (G1, G2 and G3). The serum globulin increased in the first and fourth groups of treatment (G1 and G4) from the 1st to 7th day of treatment compared with the 0-day, and from the 5th and 7thday in the second and third groups of treatment compared with the 0-day. These results agreed with Herz and Kirberger (2004) and Nagata and Yuki (2013). This result could be attributed to the improvements of diet, liver, kidney function and protein metabolism and decreased inflammation of joint and bones that was decreased stress and pain so increase willing and ability of cats to move and eat much.

Calcium level revealed a significant increased from the 1st day of treatment compared with 0-day in all groups of treatment. This result agreed with Moarrabi et al. (2008), Dimopoulou et al., (2010), Mackenzie *et al* (2011), Pineda *et al*(2012), and Nagata and Yuki (2013) .This result could be attributed to the increase calcium intake, improvement of albumin level that results in increased calcium level, and balanced diet the cat received with the treatment. On the other hand, phosphorus level was gradually decreased beginning from the 3rd day of treatment in the first, second and third groups of treatment (G1, G2 and G3) but began from the 1st day of treatment in the fourth group of treatment (G4). This result agreed with Moarrabi *et al*. (2008), Mackenzie *et al*. (2011), Pineda *et al*. (2012), Nagata and Yuki (2013). This result could be attributed to decreased phosphorus intake by offering balanced diet and the increased calcium intake(calcium preparation) in the 4 treatment groups.

Magnesium level revealed significant increase from the 3rd day of treatment in all treatment groups with no significant changes between treatment groups all over the period of treatment. This result agreed with Robert and Rude (1998). This result could be attributed to decreased Mg urinary excretion, cessation of mobilization of Mg from bone and increased Mg dietary source. It could also be attributed to decreased PTH because of some metabolic studies shoe a negative Mg balance I in hyperparathyroidism. On the other hand, the copper level was significantly increased from the 3rd day of treatment in all groups of treatment with. Similarly, zinc level revealed significant increase from the 3rd day of treatment in the second, third and fourth groups of treatment but from the 5th day of treatment in the first group of treatment) compared with the 0-day with non-significant changes between groups of treatment all over the period of treatment. This result agreed with Robert and Rude (1998), Strain(2004). This result could be attributed to decreased Cu and Zn urinary excretion and increased Cu and Zn dietary source via the balanced diet.

5. CONCLUSION:

Based upon the results of this work, we can conclude that NSHPT can be induced in cats that fed mainly meat diets. Advanced cases with severe skeletal deformities and multiple fractures can be difficult to restore, but cases presenting mainly with pain, lameness and minor fractures respond well to dietary therapy when properly applied. While there are many protocols for treatment of NSHPT in kittens, the provision of balanced diet s and cage rest remains the first key of treatment of NSHPT in cats. The calcium supplementation is an essential part of treatment in addition to correction of the diet by a high-quality commercial food. Eggshell powder can be used as an alternative natural therapeutic preparation in small kittens to

cure the progressive bone diseases secondary to NSHPT.

6. ACKNOWLEDGMWNT:

Special thanks for Dr. Hamada Mahmoud Youssef. Production Sector Agriculture Research Center-Agriculture Ministry for helper and assistance in the management of cases. My grateful thanks are offered to all members and workers of the Department of Animal Medicine. Fac. Vet. Med., Benha University.

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