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ASSESSMENT OF CALCIUM LEVELS IN PARATHYROID DISTURBANCES IN MATURE MALE RABBITS

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Abstract

In rabbits, the absorption of calcium is directly correlated with the quantity consumed in the meal, rather than being regulated based on metabolic requirements, and absorption of calcium is relatively-independent from vitamin D3 so this research aims to assist calcium levels in parathyroid gland disorders in rabbits. Fifty mature-male-rabbits were randomly assigned into 5 groups, (10 rabbits/group); Group-1:(Control) was intraperitoneal (I/P) injected normal saline (one ml /kg /day for forty five days). Group-2: (Hyperparathyroidism); was IP injected with Lithium Chloride (72mg/kg/day for 45 days). Group-3(Hypoparathyroidism): was IP injected Omeprazole (1mg/ Kg/ for each day in 45 days). Group 4: (Calcium administered group); was IP injected with Ca2+ (96mg/ Kg/ for each day in 45 days) Group 5: (Calcium + Vit.D3 administered group); was IP injected with Ca2+ (96mg/kg/day for 45 days) I/P injection and vitamin D3 (10000IU/ Kg/ for each day in 45 days) orally. Collection of blood samples were used cardiac puncture for the biochemical, hormonal assessment [Parathyroid hormone (PTH), vitamin D3, serum calcium, phosphate and magnesium. The data of results appear considerable increase in Parathyroid hormone and Ca2+ levels in hyperparathyroidism group and significantly decrease in hypoparathyroidism group as compared with control group, in addition to that significant decrease in Mg2+ concentration in hypoparathyroidism group. In conclusion in spite of the Ca2+ have a unique mechanism for absorption in rabbits, but remain the PTH essential to control Ca2+ levels**.**

Keywords: *Calcium, Parathyroid hormone (PTH), Phosphorus, Magnesium.*

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Introduction

Calcium (Ca^{2+}) is the most abundant metal divalent ion and the most important component of the human body, especially bones and teeth **(Eckermann-Ross, 2008)** . Calcium ions; as electrolytes, are essential for a wide variety of cellular, organismal, and biochemical processes, including signal transduction (as a second messenger), neurotransmitter release from neurons, muscle contraction across all cell types, enzyme co factorization, and even fertilization **(Bradshaw & Dennis, 2009)**. Calcium ions outside of cells are required for maintaining the membrane potential across excitable cells, protein synthesis, and bone formation **(Mazzanti *et al.*, 2019)**.

The extracellular fluid calcium content is tightly controlled, with a normal value of around8.5-10.5 mg/dl in human **(Hall & Hall, 2020)**; Ca^{2+} equilibrium is determined by the net intake and outflow of Ca^{2+} by the gastrointestinal tract and kidneys. dihydroxycholecalciferol; commonly known as $1,25(OH)_2D_3$-regulated Ca^{2+} active transport from the intestine-lumen to the blood compartment, is the most essential underlying mechanism **(Müller *et al.*, 2000)**.

Both passive paracellular transport and active transcellular transport allow Ca^{2+} to (re)enter the extracellular fluid **(Van Abel *et al.*,2005)**. Transported by Paracellular way is the movement of molecules between epithelial cells that are next to one another. Transport over the narrow intersection (tight junction) is a passive process that governs the rate-determining stage of the process **(Bouillon *et al.*, 2003)**. The passage of ions across tight junctions is mostly by Ion concentration gradient permeability and trans epithelial electrical gradient. Hormones and factors that alter the electrochemical gradient across the epithelium have an indirect effect on passive fluxes via tight junctions. Under diverse physiological situations, tight junction permeability is dynamically controlled **(Goodenough, 1999)**. Many factors that play a role in modifying tight junctions such as Growth factors, cytokines, bacterial toxins, hormones, and other factors **(Gopalakrishnan *et al.*,2002; Benais-Pont *et al.*, 2003)**. According to several researches, phosphorylation for tight-junction-proteins aids in the composition and operation the tight junctions. Protein-kinase-C (PKC) modulation disables epithelial cell, cell to cell junctions, which action is believed to be mediated by mitogen-activated protein kinase (MAPK) **(Wang *et al.*, 2004)**.

Calcium transcellular-transport is a process of multi-step that begins with the absorption of luminal Ca^{2+} By intestinal cell or epithelial cell of kidney, continues with it passes through the cell to the basolateral-membrane, and ends with its extrusion actively into the blood circulation **(Van Abel *et al.*, 2005)**. Transient receptor of potential cation-channels (TRPV5) (formerly known ECaC1); an epithelial Ca^{2-}channel, provided the first molecular evidence for the existence of an apical Ca^{2+} entry route (Hoenderop *etal.*, 1999) and also TRPV6 (or called Ca^{2+} transporter-1) has been determined **(Montell *et al.*,2002)**. Since rate limiting step in active Ca^{2+}transport is transmitted by these channels, they are of critical importance in maintaining normal Ca^{2+} homeostasis (van Abel *et al.*, 2003) Ca^{2+} flow across the membrane of cytoplasm is crucial to numerous Physiological operations, making

it crucial to tightly regulate the TRPV5,TRPV6 as expression and activity by several variables such as vitamin D_3, dietary Ca^{2+}, and estrogen in order to maintain the extracellular Ca^{2+} equilibrium **(Hoenderop *et al.*, 2002)**.

Parathyroid hormone disorders are including functional abnormalities (hyper- and hypoparathyroidism) and neoplasms of the parathyroid glands are the most common parathyroid disorders **(Al-Mahdawi *et al.*, 2020; Mohan, 2015)**. Primary hyperparathyroidism is rather prevalent, especially as people get older. It occurs more frequently in postmenopausal women. About 80% of instances of primary hyperparathyroidism are caused by parathyroid adenomas, 2% to 3% are caused by parathyroid cancer, and 15% are caused by primary hyperplasia (usually chief cell hyperplasia) **(Silverberg & Bilezikian, 2006)**. This category extends to cover family instances of multiple endocrine neoplasia (MEN) syndromes in which parathyroid adenoma or primary hyperplasia is a presenting symptom **(Mohan, 2015)**. Elevated parathyroid hormone levels, hypercalcemia, hypophosphatemia, and hypercalciuria are essential biochemical manifestations of primary hyperparathyroidism **(Marques *et al.*, 2011)**.

Secondary hyperparathyroidism is caused by an increase in parathyroid hormone production as a result of another ailment in the body. Hypocalcemia produces secondary hyperparathyroidism by stimulating compensatory hyperplasia of the parathyroid glands **(Mohan, 2015)**. However, the excessive release of parathyroid hormone is the underlying cause of any illness that results in hypocalcemia; like chronic renal failure leading to phosphate retention and decreased intestinal calcium absorption, Inadequate vitamin D_3 levels can causes to rickets and osteomalacia, which in turn cause hyper-function in the parathyroid glands and intestinal malabsorption syndromes **(Ballinger *et al.*, 2014)**.

Rabbits have developed a distinctive approach wherein the majority of calcium in diet is assimilated in gut. Nevertheless, the absorption of calcium occurs in direct correlation with the quantity consumed in the meal, rather than being regulated by metabolic requirements. Additionally, the absorption of calcium in rabbits is essentially unaffected by the presence of vitamin D_3 **(Harcourt-Brown, 2004)**, So, the aim of this study is to assist Ca^{2+} homeostasis, in spite of there is a unique Ca^{2+} absorption mechanism through parathyroid gland disorders.

Methodology:

Experimental Animals

This experiment was done in the College of Medicine, Basrah University in animal house, with 50 mature male rabbits weighing (1100-1200 gm) purchased from a local market in the Basrah district. Animals were kept in standard cages at 25 ± 2°C for twelve hours. Every day, there is a light/dark cycle, as well as unlimited food and drink. The animals spent two weeks acclimating in the animal-house.

The Ethical Committee of the College of Veterinary-Medicine, Basrah University, accepted the study protocol, which was carried out between January and August 2022.

Experiment Design

Fifty mature-male-rabbits, they were randomly division into five groups, (ten animals / group); Group 1 (Control or standard): was intraperitoneal (I/P) injected normal saline (one ml /kg /day for forty five days). Group 2: (Hyperparathyroidism); was IP injected with Lithium Chloride (72mg/kg/day for 45 days). Group 3: (Hypoparathyroidism); was IP injected with Omeprazole (PPI) (1mg//kg/day for 45 days). Group 4: (Calcium administered group); was IP injected with Ca^{2+} (96mg/kg/day for 45 days) Group 5: (Calcium +Vit.D3 administered group); was IP injected with Ca^{2+} (96mg/ kg /day for forty five days) I/P injection and vitamin D3 (10000IU/ kg /day for forty five days) orally.

***Note: Dosages of drugs administered according to* Robb (2008).**

Blood Sample Collection

Samples were taken at the conclusion of experiment by cardiac puncture using a disposable-syringe (5 cc), and then placed in a plan tubes and centrifugation at (3000 rpm /15 min.) for extract serum, and then putting in tubes of Eppendorf and kept in (-20C°) for use in various hormonal and biochemical parameter investigations.

Laboratory analysis

The BT LAB sandwich kit (Biological Testing Technology, China, cat. no. E0195Rb/2022) used for the precise-quantitative for detection of serum rabbit parathyroid hormone.; ELISA test kit **(Shimizu *et al.*, 2002)**. While the quantification of serum Vit. D_3 used Bioassay Technology Laboratory, competitive ELISA kit (BT LAB, China, Cat. NO. EA0043Ge, 2020) **(Heijboer *et al.*, 2012)**. The calcium concentration in the blood was measured at 450 nm using a kit commercially available from BioSystems (Spain) **(Qubih, 2012)**. While the phosphorus concentration was determined using a proprietary BioSystems (Spain) phosphorus kit (Burtis & Bruns, 2014), and a QuantiChromTM Magnesium Assay Kit (DIMG-250, Bioassay Systems, USA, 2021) used to determine the serum magnesium concentration. Magnesium quantification via colorimetric analysis at 500 nm **(Elizondo *et al.*, 2010)**.

Statistical analysis

All recorded and computed data were analyzed using the SPSS (Version 26) for ANOVA-analysis one way utilizing complete randomized-design (CRD). The information was presented as (mean ± standard deviation) (M±SD). $P<0.05$ was deemed significant**(Petrie & Watson, 2013)**.

Results

The data of results for PTH concentration revealed a significantly increment (P≤0.05) in hyperparathyroidism group(8.12±1.02 pg/ml). While, the PTH concentration significant decrease (P≤0.05) in hypoparathyroidism group (2.34±0.80 pg/ml), and in Ca^{2+}+Vit-D_3 administration group (2.12±0.95 pg/ml); in comparison to the control and other treated groups. On other hand significantly (P≤0.05) increase in Vit-D_3 hormone in Ca^{2+}+Vit-D_3 administration group (91.19±19.69 ng/ml) than other treated and control groups(Table 1). While, a significant(P≤0.05) increase of Ca^{2+} concentrations in hyperparathyroidism group (16.38±0.71 mg/dl), and the results of Ca^{2+}+ vit-D_3 administration group (15.30±0.69 mg/dl), which elevation significantly (P≤0.05) in their Ca^{2+} concentrations after forty five days of treatment, as compared to other treated and control groups. Additionally, there is significantly decrease (P≤0.05) of Ca^{2+} concentrations in hypoparathyroidism group (13.46±0.94 mg/dl) compared with control group, and the level of PO_4^{3-} concentration significantly decreased (P≤0.05) in hypoparathyroidism group (2.09±1.63 mmol/L) as compared to all treated groups. While the Mg^{2+} concentrations are significantly decrease (P≤0.05) in the hypoparathyroidism-group as compared with all groups (Table 2).

Tab.1: The effects of hyper/hypoparathyroidism, $Ca^{2+}$and Ca^{2+}+Vit-D_3 administration on PTH, Vit-D_3 hormones in mature-male-rabbits (M±SD).

Parameters. / Groups.	PTH (pg/ml)	Vit.D_3 (ng/ml)
Control Group	5.70±3.32A	23.57±2.34B
Hyper PTH Group	8.12±1.02A	18.27±3.26C
Hypo PTH Group	2.34±0.80B	9.05±3.46C
Ca^{2+} Admin. Group	5.87±3.63A	7.98±3.73C
Ca^{2+} & Vit-D_3 Group	2.12±0.95B	91.19±19.69A
LSD	3.35	14.52

Capital-letters signify significant-differences-between groups (P<0.05) vs to control-group.

Tab.2: The effects of hyper/hypoparathyroidism, Ca^{2+} and Ca^{2+}+ Vit-D_3 administration on Ca^{2+}, PO_4^{3-}, and Mg^{2+} concentrations in mature-male-rabbits (M±SD).

Parameters. / Groups.	Ca^{2+} (mg/dl)	PO_4^{3-} (mmol/L)	Mg^{2+} (mg/dl)
Control Group	14.16±0.39C	4.64±1.86A	3.71±0.35B
Hyper PTH Group	16.38±0.71A	3.83±0.97B	3.76±0.43B
Hypo PTH Group	13.46±0.94C	2.09±1.63B	2.83±0.52B
Ca^{2+}Admin. Group	14.92±0.88C	4.77±1.81A	4.11±0.41A
Ca^{2+} & Vit-D_3 Group	15.30±0.69AB	4.02±0.62AB	4.00±1.07A
LSD	1.08	2.21	0.95

Capital-letters signify significant differences between groups ($P<0.05$) vs to control-group.

Discussion

Calcium is a necessary mineral that is required for numerous physiological processes such as bone and tooth development, nerve transmission, function of muscle, and coagulation of blood. The regulation of calcium metabolism is a complex process that involves several hormones (PTH, Calcitonin and Vitamin D_3) and organs (kidney, intestine and bone) **(Sabeeh & AL-Saeed, 2020)**. In this study; the findings revealed a statistically-significant elevation in serum parathyroid hormone (PTH) levels within the hyperparathyroidism-group, comparison to all other groups. The observed outcomes might perhaps be attributed to the lithium direct effects on the parathyroid-glands, where it action like an antagonist to the Ca^{2+} sensing-receptor (CaSR). This action of lithium raises the threshold of calcium required to lower parathyroid hormone (PTH) production, thereby lessening the inhibitory effect of blood calcium on PTH **(Lerena *et al.*, 2022)** , Additionally, the long-term administration of lithium medication has been found to induce permanent modifications in parathyroid-glands, leading to development of multiglandular hyperparathyroidism. So, the biochemical profile of the latter disease is indistinguishable from that of primary hyperparathyroidism **(Dwight *et al.*, 2002)**. These findings have been agreed with a number of studies of lithium induced hyperparathyroidism in humans and animals **(Bas *et al.*, 2005; Livingstone & Rampes, 2006b and Lehmann & Lee, 2013b)**. In additionally, to that this study findings a highly significant-decrease in PTH level in the hypoparathyroidism-group, which agreed with previous findings of **Adam *et al.*, (2020)**; who reported that severe-hypomagnesemia is produced by PPI **(Swaminathan, 2015)**; that

influenced the ability of parathyroid gland to release the effect of PTH and PTH on target organs by inhibiting cAMP production, which requires magnesium, This resulted in decreased reabsorption of calcium from the kidneys, decreased release of calcium from the bones via parathyroid hormone, and decreased synthesis of vitamin D3, which resulted in decreased absorption of calcium from the intestine. I disagree with that result of **Chowdhry *et al.*, (2018)** that determined that proton pump inhibitors had no effect on magnesium levels.

On other hand significant increase in Vit-D_3 hormone in Ca^{2+}+Vit-D_3 administration group; VitD_3 level is importance to maintain the balance of calcium, phosphorus and ultimately bone metabolism **(Kang *et al.*, 2017)**, so the PTH concentration in relation to 25 $(OH)_2D_3$ level serve commonly to determine VitD_3 deficiency-levels **(Sai *et al.*, 2011)**; Because Ca^{2+}absorption in intestines of humans and other animals is Vit D-dependent, Vit-D deficiency seems to lower blood Ca^{2+}concentration, resulting in increment PTH-levels. In addition to, 25 $(OH)_2D_3$-concentration, at which the PTH concentration started to rise or stops falling, may be indicated to Vit D-inadequacy **(Del Valle *et al.*, 2011)**. In rabbits, the absorption of Ca^{2+} is primarily passive Although elevated PTH concentration occurred more frequently in rabbits with vitamin D deficiency than in the control-group., and these results agreement with **Kang *et al.* (2017)** whom concluded to the serum PTH decreased continuously as serum-25 $(OH)_2D_3$ increased (6 to 60 ng/ml).

The results obtained in the current study revealed an increase in Ca^{2+} concentration in hyperparathyroidism group, but not significantly difference; because high level of PTH promotes osteoclast activity within the bone, causing calcium release to extracellular fluid and PTH stimulation the calcium reabsorption and inhibition the phosphate-reabsorption from the tubules in the kidneys, as well as stimulating the production of vitamin D_3 which increased the Ca^{2+} absorption from GIT **(Amerman, 2020; Sadiq, 2021)**. The results in this study is in agreement with those of **Bai *et al.* (2006); Bandeira *et al.* (2013) and *Bilezikian et al.* (2022)** they concluded that consequent hypercalcemia ordinary results of hyperparathyroidism. Furthermore, Mg^{2+} concentrations are lowering significantly in hypoparathyroidism group compared to all other treated and control groups; hypomagnesemia caused by omeprazole and other proton pump inhibitors is caused by insufficient absorption of Mg^{2+} Intestine and an imbalance between passive and active transport in the intestinal lumen due to intestinal pH changes that alter the function of the duct (active transport) or due to exposure of sensitive patients to harm **(Pérez, 2012)** and these finding agreed with **Florentin & Elisaf (2012); Delgado *et al.* (2013)** They found that the dose-responses between the use of proton pump inhibitors (PPI) and the hypomagnesemia development.

In conclusion, Ca^{2+} levels either hypercalcemia or hypocalcemia; remain depended on PTH concentration, in spite of the Ca^{2+} have a unique mechanism for absorption in rabbits.

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