Endocrine (2021) 72:234–238
https://doi.org/10.1007/s12020-020-02504-0

ORIGINAL ARTICLE

Could there be a role of serum zonulin increase in the development of hypercalcemia in primary hyperparathyroidism

Hakan Korkmaz[1] · Fevziye Burcu Sirin[2] · Bora Torus[3]

Received: 3 June 2020 / Accepted: 17 September 2020 / Published online: 28 September 2020

Abstract

Purpose To evaluate the serum level of zonulin, which is an intestinal permeability (IP) biomarker, in primary hyperparathyroidism (PHPT) and to investigate the relationship between zonulin, calcium, and parathormone (PTH) levels.

Methods The study included 34 healthy control (HC) and 39 patients with PHPT. Serum calcium, phosphorus, magnesium, creatinine, albumin, and 24 h urine calcium levels were measured in all groups. Serum levels of zonulin were measured quantitatively by enzyme-linked immunosorbent assay (ELISA). Urinary ultrasonography (to assess the presence of nephrolithiasis) and dual energy X-ray absorptiometry (to assess the presence of osteoporosis) were used to evaluate complications related to PTHP.

Results Serum zonulin levels were significantly higher in the PHPT group than the HC group ($p < 0.001$). Zonulin levels were significantly positively correlated with plasma PTH and serum calcium levels ($r = 0.600$, $p < 0.001$ and $r = 0.610$, $p < 0.001$; respectively). There was no correlation between serum zonulin levels and adenoma volume.

Conclusion Serum zonulin level increases in patients with PHPT. Serum zonulin levels show a moderate/strong positive correlation with serum calcium and plasma PTH levels. This suggests that IP increase may play a role in the development of hypercalcemia in patients with PHPT.

Keywords Hypercalcemia · Intestinal permeability · Primary hyperparathyroidism · Zonulin

Introduction

Primary hyperparathyroidism (PHPT) is an endocrine disease characterized by an increase in serum calcium levels together with parathormone (PTH). Approximately 80% of the time, disease is caused by a single parathyroid adenoma [1].

Mechanisms of hypercalcemia in PHPT are increased calcium release from bone to blood, and increased calcium absorption from renal distal tubules and intestines. The role of PTH in the regulation of renal epithelial calcium transport is well known, but there are uncertainties about the effect of PTH on intestinal calcium transport. The generally accepted view is that PTH increases the production of 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$] and indirectly leads to an increase in intestinal calcium absorption through $1,25(OH)_2D_3$ [2–4]. However, the detection of PTH-related peptide receptor type 1 (PTHR1) in rat duodenal enterocytes suggests that PTH may also have a direct effect on intestinal calcium transport [5].

Intestinal calcium absorption is important to maintain calcium homeostasis, and this absorption occurs through paracellular and transcellular transport. These transport mechanisms are regulated by hormones, nutrients, and various factors which are being investigated. The transcellular pathway is mainly regulated by vitamin $1,25(OH)_2D_3$. This regulation occurs by stimulating the transient receptor potential vanilloid 6 (TRPV6), calbindin-D9k, and the basolateral membrane calcium ATPase (PMCA1b) in enterocytes [6, 7].

The intestinal paracellular pathway is regulated by tight junctions (TJs) located between enterocytes. TJs play an

✉ Hakan Korkmaz
drhkorkmaz@yahoo.com.tr

[1] Department of Internal Medicine, Division of Endocrinology, Faculty of Medicine, Suleyman Demirel University, Cunur, 32260 Isparta, Turkey

[2] Department of Biochemistry, Faculty of Medicine, Suleyman Demirel University, Cunur, 32260 Isparta, Turkey

[3] Department of Internal Medicine, Faculty of Medicine, Suleyman Demirel University, Cunur, 32260 Isparta, Turkey

important role in maintaining intestinal barrier functions [8]. The physiological modulator of intestinal TJs is zonulin. It is a biomarker used as an indicator of intestinal permeability (IP). Increased zonulin levels loosen TJs and cause an increase in IP [9–11]. It is unknown whether there is a change in IP in patients with PHPT or its contribution to hypercalcemia.

Our primary purpose in this study is to evaluate serum levels of zonulin, which is an IP indicator, in PHPT and our second aim is to investigate the relationship between zonulin, calcium, and PTH levels.

Materials and methods

Study population

G*Power software (version 3.1.9.2, Kiel, Germany) was used to determine the sample size in the study ($\alpha = 0.05$, power = 0.90). The study included 39 patients with PHPT (median age 51.15 ± 14.14; 23 female, 16 male) and 34 healthy controls (HC) (median age 54.97 ± 6.28; 14 female, 20 male). In the PHPT group, inflammatory bowel disease, malignancy, infectious disease, use of any medication or vitamin D supplements, pregnancy, kidney failure, liver failure, familial hypocalciuric hypercalcemia, and secondary hyperparathyroidism were determined as exclusion criteria. The exclusion criteria for the HC group were determined as having any systemic disease and using any medication.

The diagnosis of PHPT was made based on the presence of normal or high serum PTH levels inappropriately in case of hypercalcemia [12]. To localize parathyroid adenoma, neck ultrasonography (US) was performed for all patients using high-resolution US (Philips Healthcare EPIQ 5 Ultrasound System Inc, Germany) with a 13 MHz linear array transducer. The volume of parathyroid adenoma was calculated by the ellipsoid model formula (length × thickness × width × 0.52) [13]. Technetium 99 m methoxyisobutylisonitrile (Tc-99m MIBI) scintigraphy was performed and images were taken at 20 min and 3 h after injection.

To evaluate the complications related to PTHP, urinary US (to assess the presence of nephrolithiasis) and Dual Energy X-Ray Absorptiometry (to assess the presence of osteoporosis) were performed. Nine of the PHPT cases had osteoporosis and 12 had urolithiasis.

Biochemical analysis

After a minimum of 8 h fasting, venous blood samples were collected into two blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) and clot activator-serum separator and centrifuged at 3000 rpm for 10 min to separate plasma and serum specimens. Serum calcium, phosphorus, magnesium, alanine aminotransaminase (ALT), creatinine, albumin, and urine calcium concentrations were measured by Beckman Coulter AU 5800 chemistry analyzer (Beckman Coulter, Brea, CA, USA). The corrected calcium (cCa) was calculated using the equation: $cCa = [(4 - \text{albumin}) \times 0.8] + Ca$.

Serum 25-hydroxy vitamin D [25(OH)D] and plasma PTH concentrations were measured by electrochemiluminescence immunoassay (Elecsys Vitamin D Total and Elecsys PTH; Roche Diagnostics, Mannheim, Germany).

Serum zonulin levels were measured by using the sandwich-enzyme-linked immunosorbent assay (ELISA) kit (E-EL-H5560 Elabscience, Wuhan, Hubei Province, China). The sensitivity of the ELISA kit was 0.47 ng/ml.

Statistical analysis

Statistical analysis was done using SPPS 22.0 package program. The chi-square test was used to compare categorical data (gender). The distribution of parameters was evaluated by the Shapiro–Wilk test. Parameters showing normal distribution (age, phosphorus) were compared with Student's t test and parameters showing non-normal distribution were compared using Mann–Whitney U test. Correlations between parameters (PTH, cCa, zonulin) were evaluated with Spearman's correlation analysis. $P < 0.05$ was considered statistically significant.

Results

Demographic, clinical and laboratory parameters of the groups are given in Table 1. There was no difference in age and gender between the groups ($p = 0.151$ and $p = 0.129$, respectively). There was no difference between the groups in terms of creatinine, ALT, and 25(OH)D levels. PTH, serum cCa, and albumin levels were significantly higher in the PHPT group than the HC group ($p < 0.001$, for each all). Serum phosphorus and magnesium levels were significantly lower in the PHPT group than the HC group ($p < 0.001$ and $p = 0.010$, respectively).

Serum zonulin levels of the groups are shown in Fig. 1. Serum zonulin levels were significantly higher in the group with PHPT than in the HC group ($p < 0.001$). Serum zonulin levels were significantly positively correlated with PTH and serum cCa levels, and are shown in Figs. 2, 3 ($r = 0.600$, $p < 0.001$ and $r = 0.610$, $p < 0.001$; respectively).

In the PHPT group, the median urinary calcium excretion was 88.5 ± 53.5 mmol/L and the median parathyroid adenoma volume was 393.12 ± 1071.46 mm^3. There were no

Table 1 Clinical and laboratory characteristics of the groups

	PHPT	HC	*p*
Age (years)[a]	51.15 ± 14.14	54.97 ± 6.28	0.151
Gender (female/male)	23/16	14/20	0.129
PTH (ng/L)	146 ± 104	35.50 ± 13	**<0.001**
25(OH)D (nmol/L)	36.19 ± 32.45	49.92 ± 32.45	0.177
Creatinine (mmol/L)	69.84 ± 16.8	73.68 ± 22.1	0.162
ALT (U/L)	21.5 ± 10	18.5 ± 10	0.161
cCa (mmol/L)	2.85 ± 0.27	2.33 ± 0.16	**<0.001**
Phosphorus (mmol/L)[a]	0.85 ± 0.16	1.08 ± 0.11	**<0.001**
Magnesium (mmol/L)	0.84 ± 0.09	0.86 ± 0.16	**0.010**
Albumin (g/L)	43.4 ± 5.5	40 ± 3.0	**<0.001**
Zonulin (ng/ml)	209.37 ± 43.72	115.31 ± 121.88	**<0.001**
Urinary calcium excretion (mmol/day)	88.5 ± 53.5		
Volume of parathyroid adenoma (mm^3)	393.12 ± 1071.46		
Urolithiasis	12/27		
Osteoporosis	9/30		

Datas expressed as median (±IQR)

PTH parathormone, *25(OH)D* 25-hydroxy vitamin D, *cCa* corrected calcium, *ALT* alanine aminotransaminase, *PHPT* primary hyperparathyroidism, *HC* healthy controls

[a]Mean (±SD)

Bold values are statistically significant ($p < 0.05$)

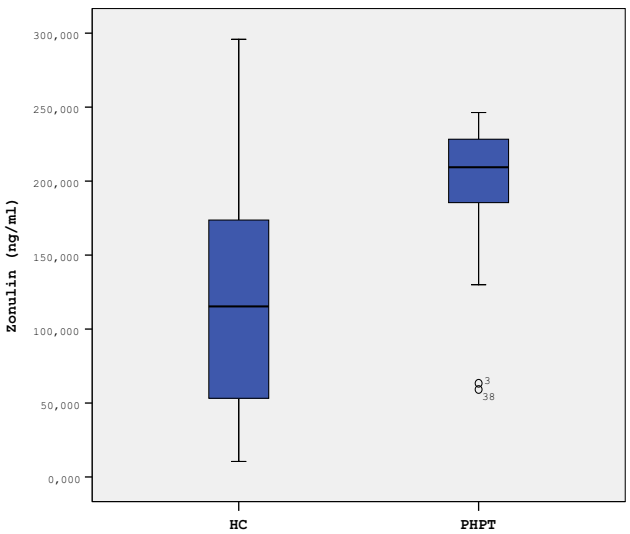

Fig. 1 Serum zonulin levels in PHPT and HC groups

correlations between serum zonulin levels with urinary calcium excretion and adenoma volume ($r = 0.258$, $p = 0.186$ and $r = -0.244$, $p = 0.178$; respectively).

Discussion

The current study is the first study to show that serum zonulin levels increase in patients with PHPT. In addition,

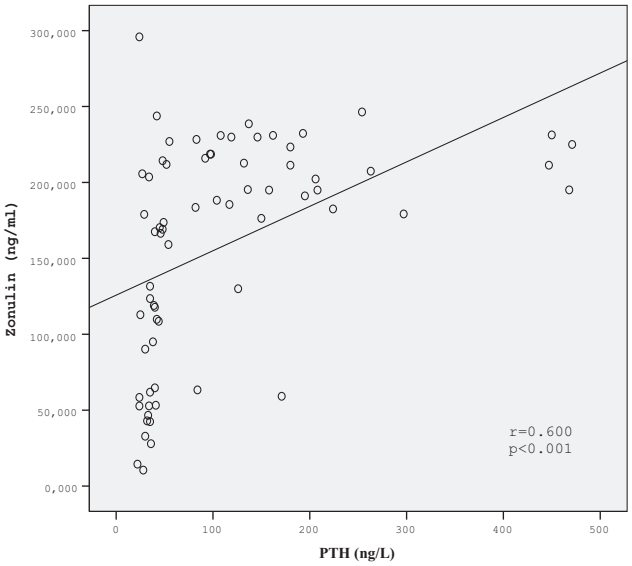

Fig. 2 Correlation between zonulin and PTH levels

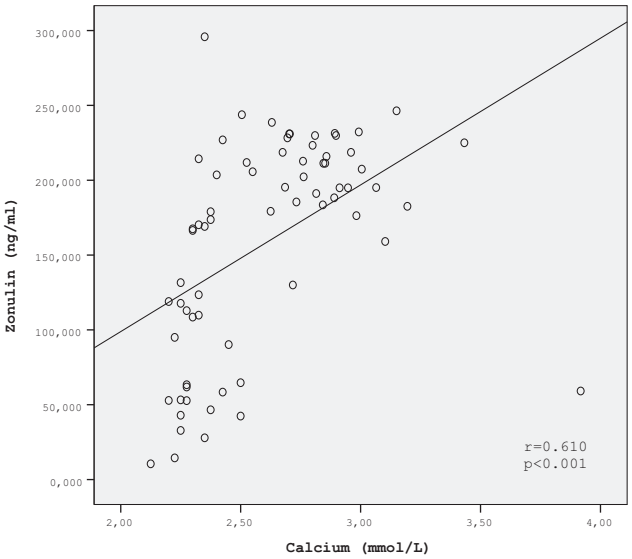

Fig. 3 Correlation between serum zonulin and cCa levels

increased serum zonulin levels were positively correlated with PTH and serum cCa levels.

Intestinal paracellular absorption of calcium is regulated by TJs located between enterocytes. TJ is composed of several transmembrane and cytosolic proteins, including zonula occludens (ZOs), occludin (Ocl), claudins, cingulin, tricellulin, and junctional adhesion molecules [14]. These proteins close intercellular junctions and limit the free movement of ions through the paracellular space. Claudin-2, -12, and -15 were shown to play important roles in the regulation of calcium intestinal paracellular transport. The roles of ZO-1 and Ocl in paracellular transport are not clear [15, 16]. The degradation of TJs structure affects IP and causes the absorption of some minerals to be impaired [17].

Whether there is a change in IP in patients with PHPT has never been evaluated. In this study, serum level of zonulin, which is the biomarker of IP, was higher in patients with PHPT than in HC. Studies have shown that vitamin D regulates IP by restoring ZO-1 and claudins in the structure of intestinal TJs. IP decreases in the case of vitamin D deficiency [18–20]. In the current study, patients with PHPT had vitamin D deficiency. Also, there was no significant difference between PHPT and HC groups in terms of 25(OH)D levels.

The increase in signal activation of $1,25(OH)_2D_3$-vitamin D receptor (VDR) in the intestinal epithelium is thought to be responsible for intestinal calcium absorption increase in PHPT [21]. In the current study, $1,25(OH)_2D_3$ levels were not evaluated. However, studies have reported that $1,25(OH)_2D_3$ vitamin levels show a positive correlation with 25(OH)D levels, while it does not correlate with both PTH and serum calcium levels [22, 23]. In this study, zonulin level was higher in the PHPT group although there was no difference between the groups in terms of vitamin D levels. Du et al. showed that $1,25(OH)_2D_3$-VDR signal activation in gut epithelium regulates intestinal epithelial permeability by directly protecting TJs [24]. In the current study, although there was no difference between the groups in terms of vitamin D levels, zonulin level was high in the PHPT group.

In addition, in the current study, serum zonulin level did not correlate with 25(OH)D level although it showed moderate/strong positive correlation with plasma PTH level, suggesting that PTH may have a direct effect on IP increase. In a study evaluating intestinal calcium absorption in patients with PHPT, they found more intestinal calcium absorption in patients with PHPT than HC although vitamin D levels were similar. They suggested that another factor other than $1,25(OH)_2D_3$ plays an important role in intestinal calcium absorption in patients with PHPT [25]. In the current study, although there was a moderate/strong positive correlation between serum calcium and zonulin levels, there was no correlation with urinary calcium excretion. It suggests that IP increase may be an important factor in the development of hypercalcemia in PHPT. However, there is not enough evidence to make this conclusion as it is a cross-sectional study. Further prospective studies are needed to clarify this situation.

The current study is important because it will bring a different perspective to the pathogenesis of hypercalcemia developing in PHPT. However, it has some limitations. Firstly, causal inferences could not be made as the design of the study was cross-sectional. Secondly, the number of cases included in the study was relatively low and a standard diet was not applied to the participants. Thirdly, fecal zonulin levels could not be evaluated.

In conclusion, serum level of zonulin, an IP biomarker, increased in patients with PHPT in the current study. Serum zonulin levels show a moderate/strong positive correlation with PTH and serum cCa levels, which suggests that IP increase may play a role in the development of hypercalcemia. We believe clear understanding of the intestinal paracellular calcium transport mechanism will lead to changes in treatment approaches for hypercalcemia in patients with PHPT.

Author contributions H.K. was responsible for the design, management, data collection, analysis, and writing of the study. F.B.S. was involved in the design of the study and biochemical measurements. B. T. was responsible for data collection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted according to the principles of the Helsinki Declaration after it was approved by the ethical committee of Medical Faculty of Suleyman Demirel University (decree number:129). All participants were informed about the research protocol, and they declared their voluntary attendance by signed written consent.

Informed consent Written informed consent was obtained from all patients.

References

1. K.L. Insogna, Primary hyperparathyroidism. N. Engl. J. Med. **379**, 1050–1059 (2018)
2. K. Yonemura, H. Suzuki, Y. Fujigaki, A. Hishida, New insights on the pathogenesis of hypercalcemia in primary hyperparathyroidism. Am. J. Med. Sci. **320**, 334–336 (2000)
3. B. Ko, Parathyroid hormone and the regulation of renal tubular calcium transport. Curr. Opin. Nephrol. Hypertens. **26**, 405–410 (2017)
4. D. Goltzman, Physiology of parathyroid hormone. Endocrinol. Metab. Clin. N. Am. **47**, 743–758 (2018)
5. C. Gentili, S. Morelli, A.R. de Boland, Characterization of PTH/PTHrP receptor in rat duodenum: effects of ageing. J. Cell. Biochem. **88**, 1157–1167 (2003)
6. G. Diaz de Barboza, S. Guizzardi, Tolosa, N. de Talamoni, Molecular aspects of intestinal calcium absorption. World J. Gastroenterol. **21**, 7142–7154 (2015)
7. A.V. Pérez, G. Picotto, A.R. Carpentieri, M.A. Rivoira, M.E. Peralta López, N.G. Tolosa de Talamoni, Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway. Digestion **77**, 22–34 (2008)
8. L.K. Wood Heickman, M.D. DeBoer, A. Fasano, Zonulin as a potential putative biomarker of risk for shared type 1 diabetes and celiac disease autoimmunity. Diabetes Metab. Res. Rev. (2020). https://doi.org/10.1002/dmrr.3309
9. A. Fasano, All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F1000Res (2020). https://doi.org/10.12688/f1000research.20510.1

10. P. Singh, J. Silvester, X. Chen, H. Xu, V. Sawhney, V. Rangan et al. Serum zonulin is elevated in IBS and correlates with stool frequency in IBS-D. United Eur. Gastroenterol. J. **7**, 709–715 (2019)
11. C. Sturgeon, A. Fasano, Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers **4**, e1251384 (2016)
12. J.P. Bilezikian, L. Bandeira, A. Khan, N.E. Cusano, Hyperparathyroidism. Lancet **391**, 168–78 (2018)
13. M. Almquist, A. Bergenfelz, H. Mårtensson, M. Thier, E. Nordenström, Changing biochemical presentation of primary hyperparathyroidism. Langenbecks Arch. Surg. **395**, 925–928 (2010)
14. C. Chelakkot, J. Ghim, S.H. Ryu, Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp. Mol. Med. **50**, 103 (2018)
15. J.L. Roxas, V.K. Viswanathan, Modulation of intestinal paracellular transport by bacterial pathogens. Compr. Physiol. **8**, 823–842 (2018)
16. K. Wongdee, M. Rodrat, J. Teerapornpuntakit, N. Krishnamra, N. Charoenphandhu, Factors inhibiting intestinal calcium absorption: hormones and luminal factors that prevent excessive calcium uptake. J. Physiol. Sci. **69**, 683–696 (2019)
17. H. Mineo, M. Amano, H. Chiji, N. Shigematsu, F. Tomita, H. Hara, Indigestible disaccharides open tight junctions and enhance net calcium, magnesium, and zinc absorption in isolated rat small and large intestinal epithelium. Dig. Dis. Sci. **49**, 122–132 (2004)
18. C. Lee, E. Lau, S. Chusilp, R. Filler, B. Li, H. Zhu et al. Protective effects of vitamin D against injury in intestinal epithelium. Pediatr. Surg. Int. **35**, 1395–1401 (2019)
19. M. Ghareghani, R.J. Reiter, K. Zibara, N. Farhadi, Latitude, vitamin D, melatonin, and gut microbiota act in concert to initiate multiple sclerosis: a new mechanistic pathway. Front. Immunol. **9**, 2484 (2018)
20. A. Assa, L. Vong, L.J. Pinnell, N. Avitzur, K.C. Johnson-Henry, P.M. Sherman, Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation. J. Infect. Dis. **210**, 1296–1305 (2014)
21. M.D. Walker, J.P. Bilezikian, Vitamin D and primary hyperparathyroidism: more insights into a complex relationship. Endocrine **55**, 3–5 (2017)
22. J.L. Shaker, K.W. Krawczyk, J.W. Findling, Primary hyperparathyroidism and severe hypercalcemia with low circulating 1,25-dihydroxyvitamin D. J. Clin. Endocrinol. Metab. **71**, 1305–1309 (1990)
23. R.V. Thakker, L.J. Fraher, S. Adami, R. Karmali, J.L. O'Riordan, Circulating concentrations of 1,25-dihydroxyvitamin D3 in patients with primary hyperparathyroidism. Bone Min. **1**(2), 137–144 (1986)
24. J. Du, Y. Chen, Y. Shi, T. Liu, Y. Cao, Y. Tang et al. 1,25-Dihydroxyvitamin D protects intestinal epithelial barrier by regulating the myosin light chain kinase signaling pathway. Inflamm. Bowel Dis. **21**, 2495–2506 (2015)
25. M. Hino, I. Yamamoto, C. Shigeno, J. Aoki, S. Dokoh, M. Fukunaga et al. Evidence that factors other than 1,25 dihydroxyvitamin D may play a role in augmenting intestinal calcium absorption in patients with primary hyperparathyroidism. Calcif. Tissue Int. **38**, 193–196 (1986)