Ghrelin Levels in Patients with Rickets

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ABSTRACT

Rickets is a common metabolic bone disease in infants. The predominant cause of rickets is vitamin D deficiency. Vitamin D is required for bone formation. The role of ghrelin in bone metabolism is unclear, but it may have an indirect effect. There are also reports in the literature suggesting that ghrelin acts directly on osteoblasts as a positive regulator. To evaluate the relationship between ghrelin levels and the development of rickets. We evaluated the relationship between serum/ saliva ghrelin levels and rickets in a cohort of 10 patients aged 6-24 months with nutritional rickets and completely healthy 14 children. Plasma ghrelin levels were measured using a commercial radioimmunoassay kit. The saliva ghrelin measurements were performed using a Human-ghrelin- radioimmunoassay-sensitive kit. Ghrelin levels in blood and saliva were found significantly lower in the rickets group compared to the healthy subjects in this study. Findings of this study shows that there is a relationship between ghrelin and the development of rickets. Decreased ghrelin levels may have caused to poor infant appetite and impaired nutritional status. Furthermore the absence of ghrelin's calciotropic effects may have contributed to the development of rickets. Detailed studies with larger series are warranted to support these findings.

Key words: *Ghrelin, rickets, vitamin D*

Riketsli Hastalarda Ghrelin Düzeyleri

ÖZET

Rikets erken çocukluk döneminde sık rastlanan metabolik bir kemik hastalığıdır. Başlıca sebebi vitamin D eksikliğidir. Vitamin D kemiklerin oluşumu için gereklidir. Kemik metabolizmasında ghrelinin rolü açık değildir ancak dolaylı yoldan etkisi olabilir. Ayrıca literatürde ghrelinin osteoblastlar üzerine doğrudan pozitif bir düzenleyici olarak etki ettiğine dair yayınlar vardır. Ghrelin düzeyleri ile rikets gelişimi arasındaki ilişkiyi incelemek. Nutrisyonel riketsi olan, yaşları 6- 24 ay arasında değişen 10 hasta ve 14 sağlıklı çocukta serum/ tükürük ghrelin düzeyleri ile rikets arasındaki ilişkiyi inceledik. Plazma ghrelin düzeyleri ticari bir radioimmunoassay kiti kullanılarak ölçüldü. Tükürük ghrelini ölçümleri, İnsan ghrelini- radioimmunoassay- duyarlı kit kullanılarak yapıldı. Kan ve tükürük ghrelin düzeyleri, rikets grubunda çalışmadaki sağlıklı kişilere göre anlamlı olarak düşük bulundu. Çalışmanın sonuçları rikets gelişimi ile ghrelin arasında bir ilişkinin var olduğunu göstermektedir. Azalmış ghrelin düzeyleri, infantın iştahının ve nutrisyonel durumunun olumsuz yönde etkilenmesine hem de ghrelinin kemik dokusu üzerindeki kalsiyotropik etkisinin ortadan kalkmasına sebebiyet vermiş olabilir. Ancak daha fazla olguyu içeren ayrıntılı çalışmalarla bunun desteklenmesi gerekir.

Anahtar kelimeler: *Ghrelin, rikets, vitamin D*

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INTRODUCTION

Vitamin D plays a role in calcium homeostasis and in the development and maintenance of bone; it is required for the prevention of rickets, the optimization of peak bone mass, and the prevention of bone loss, and it may reduce the risk of osteoporosis-related fractures. Vitamin D aids in the mineralization of the organic matrix in bone and mediates the release of calcium and phosphate from bone to achieve mineral homeostasis (1). Rickets is a common metabolic bone disease in infants, leading to defects in bone mineralization. Vitamin D deficiency is the most common cause of nutritional rickets; a vitamin D level <20 ng/mL is defined as vitamin D deficiency (VDD). Until recently, rickets posed an important health problem in Turkey; however, its incidence decreased with the use of vitamin D prophylaxis (2, 3).

Ghrelin is a growth hormone secretagogue, and promotes bone formation (4). It is produced in and secreted mainly from the stomach, and has a variety of regulatory functions both in the brain and peripheral tissues. Emerging evidence indicates that ghrelin stimulates appetite, promotes adipogenesis, decreases energy metabolism, improves cardiovascular function, and stimulates the release of prolactin and cortisol (5, 6). Therefore, ghrelin may have an indirect effect on bone metabolism, although its exact role remains unknown (7). The role of ghrelin in the development of rickets has not yet been investigated. To date, there are no studies on ghrelin levels in patients with rickets. This study aims to investigate the relationship between plasma/saliva ghrelin levels and rickets.

MATERIALS AND METHODS

The study was approved by the Ethical Committee of Fırat University Faculty of Medicine and written informed consent was obtained from the parents of all participants. Twenty-four 6- to 24-month-old infants whose body weight and height were between the 3rd and 97th percentiles were included in the study. Group I included 10 patients with nutritional rickets and group II consisted of 14 healthy controls. Patients in both groups were selected in the same seasonal period among infants living in the same geographic environment, whose exposure to sunlight were similar. A systemic physical examination was performed on the study and control groups; both groups were tested for the presence of rickets and the diagnosis was biochemically confirmed my measuring calcium, phosphorus, alkaline phosphatase (ALP), magnesium, parathyroid hormone (iPTH), and 25-hydroxy vitamin D [25(OH)D] levels. Serum and saliva samples were obtained from all participants to measure ghrelin levels. All infants were categorized with diagnostic codes corresponding to rickets, vitamin D deficiency, hypovitaminosis D, osteomalacia, genu varum, genu valgum, craniotabes, hypocalcaemia, hypocalcaemic seizure, and tetany. Vitamin D deficiency was defined as a serum 25(OH) D level <20 ng/mL, and vitamin D insufficiency as 21–29 ng/mL. Exclusion criteria were non-nutritional causes of rickets, vitamin D metabolism disease due to genetic disorders, and hypophosphatemic rickets.

Ghrelin levels were measured over a 24-h period in both groups given three meals per day at specified times. Unstimulated saliva (5 mL) from the patients with rickets and control groups waswere collected using the previously published method (8, 9). Ghrelin levels were measured using HPLC method. Plasma ghrelin levels were measured using a commercial radioimmunoassay (RIA) kit (total ghrelin [Cat. No. GHRT-89HK]; acylated ghrelin [Cat. No. GHRA-88HK]; Linco Research, St Louis, MO, USA) according to the manufacturer's instructions. Saliva ghrelin measurements were performed using a Human-ghrelin-RIA-sensitive kit (Karlsruhe, Germany), Statistical analysis was performed using SPSS (version15.0, Chicago, IL, USA) for Windows. Correlations between two variables were tested by Pearson's correlation coefficient. The results were expressed as mean ± SD; $p<0.05$ was considered statistically significant. The statistical significance of differences between the study group and the controls was estimated by one-factor analysis of variance (ANOVA).

The authors have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals.

RESULTS

Characteristics and biochemical values of both groups are shown in Table 1. Group I included three female and seven male patients with rickets, with an average age of 9.7±3.7 months. The control group included six female and eight male healthy volunteers with an average age

***Table 1**. Characteristics of voluntary participants in both groups*

	Rickets	*Control*	*p value*
Gender (girls/boys)	*3/7*	*6/8*	*0.67*
Age (month)	*9.7±3.7*	*8.6±3.7*	*0,51*
Weight (kg)	*15.9±3.2*	*11.0±2.2*	*0.20*
Height (cm)	*90.0±26.9*	*78.8±20.4*	*0.26*

of 8.6±3.7 months. Age, weight, and height were not significantly different between the groups. There was no significant difference with regards to serum phosphorus and urine calcium excretion levels; however, there was a statistically significant difference in serum calcium, ALP, iPTH, and 25(OH)D levels between the two groups ($p<0.05$). Ghrelin levels in plasma and saliva were significantly lower in the rickets group compared to healthy controls ($p\leq0.001$) (Table 2).

DISCUSSION

There is a median fatty acid side chain on the ghrelin peptide which is required for the binding and activation of the classical ghrelin receptor. The post-translational acylation has been shown to occur independently of the proteolytic processing of the ghrelin peptide (10). The hydroxyl group of serine-3 is acylated with an n-octanoic acid or another medium-chain fatty acid, and ghrelin acylation is entirely unique to ghrelin and is required to activate the ghrelin receptor (11), the latter is referred to as active ghrelin. Non-octanoylated ghrelin does not activate type 1a GH secretagogue receptor (12, 13), and it is presumed to be inactive (11). The results of our study showed a negative correlation between ghrelin levels and rickets. In children with rickets, both active and inactive ghrelin levels in saliva and plasma were significantly lower than in the control group. Skeletal development consists of chondrocyte differentiation, longitudinal bone growth, and remodeling (14). A previous study suggested that ghrelin may have a significant role in regulating chondrocyte metabolism in the growth plate (15). Ghrelin produced by chondrocytes may influence, via an autocrine/paracrine pathway, the synthesis of factors that selectively promote osteogenesis; alternatively, ghrelin may modulate the biosynthesis of eicosanoids in cartilage (11). Similar effects have been shown in teeth formation. It has been shown that acylated ghrelin derived from chondrocytes plays an important role in chondrocyte cell biology. The expression of GHS-R1 in rat osteoblasts has been reported, indicating that ghrelin stimulates cell proliferation and differentiation. In addition, ghrelin directly promotes bone formation mediated by GHS-R1a in vivo and in vitro, and increases bone mass density in vivo; thus ghrelin is a positive regulator of osteoblasts (16). Furthermore ghrelin promotes insulin like growth factor I formation.

The data presented suggest that ghrelin signaling may be essential for normal in humans; ghrelin is a growth hormone secretagogue. Previous studies have reported a significant stimulation of osteoblast proliferation and an increased ALP activity in osteoblasts in response to ghrelin (7, 17, 18). Ghrelin stimulates growth hormone secretion both in vivo and in vitro, and may therefore have a positive effect on bone. There is also evidence for direct effects of ghrelin on bone (19). Conversely, absence of ghrelin has negative effects on bone health.

***Table 2**. Comparison of biochemical parameters of patients with rickets and the control group*

Biochemical parameters	*Rickets (n:10)*	*Control (n:14)*	*p value*
Calcium (mg/dl)	*8.3±1.3*	*10.1±0.8*	*<0.001*
Phosphorus (mg/dl)	*4.4±1.2*	*4.9±1.1*	*0.34*
ALP (U/L)	*848±296*	*261±34*	*0.029*
Parathormone (pg/ml)	*218.02±72.46*	*38.38±4.75*	*0.007*
25-hydroxy vitamin D (ng/ml)	*27.57±4.51*	*83.14±9.55*	*<0.001*
Urine Calcium/creatinine	*0.02±0.02*	*0.04±0.02*	*0.243*
Active ghrelin in blood (pg/ml)	*23.87±1.64*	*68.93±5.15*	*<0.001*
Active ghrelin in saliva(pg/ml)	*24.20±2.21*	*71.33±5.15*	*<0.001*
Inactive ghrelin in blood (pg/ml)	*117.18±18.26*	*666.14±193.73*	*<0.001*
Inactive ghrelin in saliva(pg/ml)	*101.10±3.76*	*606.14±77.76*	*<0.001*

Alkaline phosphatase activity=ALP

Bone loss as a result of ghrelin pool opening was shown in the organisms that had undergone gastrectomy (20). Lehto-Axtelius et al. (21) showed that gastrectomy/ fundectomy were associated with tibial trabecular bone loss and with reduced bone volume in both tibia and calvaria. Gastrectomy-evoked osteopenia can be reproduced by selective resection of the acid-producing part of the rat stomach.

Rickets is a childhood disease characterized by defective mineralization of growth plates and osteoid tissue in association with general osteopenia (22). Vitamin D deficiency is the predominant cause of rickets. It is associated with an increase in osteoblastic activity and possibly with serum adiponectin levels. Serum adiponectin levels have been found to be increased in rickets, and are thought to be the cause of increased osteoblastic activity (23- 25).

A previous study has shown that ghrelin is a direct positive osteoblast regulator through the direct stimulation of bone formation (7). However, no previous studies have assessed ghrelin levels in patients with rickets. The reason of the lower ghrelin levels in patients with rickets compared to controls remains unknown; it may be due to the disease or an accompanying condition. In this study, it was not possible to determine the level of ghrelin in bone and its effect, as ethical. Ghrelin deficiency in rickets may be similar to that seen in patients undergoing gastrectomy. The positive effect of ghrelin on bone tissue was eliminated in those patients. Ghrelin deficiency may have contributed to the development of rickets in our patients in a similar way.

In conclusion, this is the first study investigating the relation between serum ghrelin and rickets. The results of the study indicate that serum ghrelin levels were decreased in patients with rickets. Decreased ghrelin levels may have caused to poor infant appetite and impaired nutritional status. Furthermore the absence of ghrelin's calciotropic effects may have contributed to the development of rickets. The role of ghrelin in the regulation of bone metabolism is still largely unknown and future studies are required to better understand the relationship between ghrelin and bone.

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