

## Original Article

## Hypocalcemia in Healthy Young Girls in Tehran

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**Background:** This study was performed in an attempt to assess serum calcium, phosphorus, and alkaline phosphatase in adolescent girls and calcium supplementation in subjects with abnormal serum findings.

**Methods:** A randomized, cross-sectional, cluster sampling, prospective, and descriptive trial was conducted from January through March 2003 for measurement of serum calcium, phosphorus, and alkaline phosphatase.

The study group consisted of healthy girl students aged 11 – 15 years, from various areas of Tehran, Iran. Supplementary calcium tablets (2000 mg per day) were prescribed for 20 days for girls with low or normal serum calcium and raised alkaline phosphatase concentration.

**Results:** Of 414 girls who were evaluated, 29 (7%) had low or normal serum calcium and raised alkaline phosphatase concentration, with other normal findings. The mean serum calcium and alkaline phosphatase in these girls were  $9.4 \pm 0.4$  mg% and  $1168 \pm 156$  IU/L, respectively. The mean serum calcium in 8 (27.58%) of these 29 girls were  $8.02 \pm 0.3$  mg%. The serum calcium and alkaline phosphatase after calcium supplementation were  $9.6 \pm 0.9$  mg% and  $666 \pm 310$  IU/L, respectively, with a significant ( $P < 0.05$ ) difference existing in serum alkaline phosphatase concentration among girls before and after supplementation. The serum phosphorus concentration in all girls was normal.

**Conclusion:** Hypocalcemia is common in adolescent girls, which is among the subtle biochemical findings of rickets during the rapid growth period and may progress into symptomatic rickets.

*Archives of Iranian Medicine, Volume 9, Number 4, 2006: 364 – 367.*

**Keywords:** Alkaline phosphatase • hypocalcemia • rickets

## Introduction

Although nutritional rickets is usually attributed to vitamin D deficiency, recent reports have demonstrated that dietary insufficiency of calcium is a more common cause of rickets in Africa.<sup>1, 2</sup> A syndrome characterized by radiological, clinical, and biochemical features of rickets, with a normal

serum concentration of 25-hydroxyvitamin D [25-(OH)D], has been described in a rural black population.<sup>3, 4</sup> In this study, we decided to determine one or more serum biochemical findings of rickets, which are related to normal or abnormal serum calcium level, in an urban white healthy population living in a sunny and temperate climate. The aim of this study was to evaluate the biochemical markers of rickets in healthy girl students and therapeutic intervention in those with abnormal markers. This study protocol was approved by the Research and Ethic Committee of Shaheed Beheshti University of Medical Sciences.

## Materials and Methods

## Subjects

The study population included healthy

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Accepted for publication: 8 March 2006

adolescent guidance school (middle school) girls aged 11 – 15 years from various areas of Tehran, Iran.

Subjects were within a wide spectrum of different socioeconomic status and had no chronic or previous disease that could affect the metabolism of calcium and vitamin D.

*Phase I:* A randomized, cross-sectional, cluster sampling, prospective, and descriptive study was carried out from January through March 2003, for the detection of serum calcium and one or more abnormal biochemical findings of rickets.

After providing brief explanation on the causes, symptoms, and morbidity of rickets for parents (by letter) and obtaining the informed consent from one of the parents, the subjects were included in the study. The demographic information including the age, weight, height, and Tanner stage of all girls were recorded. Two pediatricians conducted the physical examinations, and took the history of the disease. The subjects suffering from renal failure, hepatic failure, bone disease, and malabsorption were excluded from the study. Those participants who were receiving anticonvulsant medications, or supplementary calcium and vitamin D were also excluded. No paraclinical studies were carried out to rule out the presence of chronic disease. Fasting blood samples were collected by venipuncture. After blood centrifugation, sera were separated and routine blood chemistry elements, such as serum calcium, phosphorous, and alkaline phosphatase (ALP) were analyzed by a Hitachi 717, autoanalyzer RXT Technicon system. The normal serum laboratory reference ranges of biochemical findings were defined as calcium = 8.6 – 10.6 mg%,<sup>5</sup> phosphorus = 2.5 – 5 mg%,<sup>5</sup> and ALP = 1000 IU/L.<sup>5,6</sup>

Statistical analysis was conducted using the SPSS software, version 11.5. The results were expressed as mean  $\pm$  standard deviation (SD). The significance level was set at  $P < 0.05$ . Comparison of mean values of the groups were conducted with two sample  $t$  test and among those individuals before and after intervention with paired  $t$  test.

*Phase II:* We prescribed calcium tablets 2000 mg/day (calcium forte-pars Minoo, Tehran, Iran) for those girls with low or normal serum calcium, but a raised ALP level, for 20 days. Blood samples were collected for measurement of calcium and ALP, one day after the end of the treatment.

## Results

Of over 1250 informed consents given to the

parents, we only received 414 signed parental consents. Dietary evaluation revealed that bread is the main source of dietary regime, followed by cereal, rice, meat, and vegetables. Milk and milk products were not an important source of dietary calcium intake. There were no vegetarian in this study.

Of 414 girls, 29 (7%) showed a raised ALP value. The mean ( $\pm$ SD) serum calcium in 8(27.5%) of these 29 girls were  $8.02 \pm 0.3$  mg%, ranging from 6.8 – 8.5 mg% (hypocalcemic). The serum phosphorous levels were normal in all girls, with no significant difference between normocalcemic and hypocalcemic girls. The characteristic and biochemical findings of all girls are shown in Table 1.

There were no significant difference in ALP values between normocalcemic and hypocalcemic girls ( $P > 0.05$ ). The mean  $\pm$  SD serum calcium levels before and after calcium supplementation were  $9.4 \pm 0.4$  mg% and  $9.67 \pm 0.9$  mg%, respectively, and also for serum ALP after intervention was  $666 \pm 310$  IU/L. There were significant differences in serum ALP before and after therapeutic intervention among subjects ( $P < 0.05$ ).

## Discussion

Nutritional rickets may be caused by inadequate vitamin D or calcium intake, especially during the rapid growth phases. Vitamin D is essential for the maintenance of calcium homeostasis and bone mineralization.<sup>7, 8</sup> There are numerous studies demonstrating symptomatic rickets in African and

**Table 1.** Characteristics and biochemical findings of 414 student girls.

Variable	Mean $\pm$ SD (range)
Age (yr)	13 $\pm$ 1 (11 – 15)
Weight (kg)	47 $\pm$ 11 (27.5 – 77.5)
Height (cm)	157 $\pm$ 7 (130 – 165)
Tanner stage	3.1 $\pm$ 1 (1 – 5)
Calcium (mg%)	9.4 $\pm$ 0.4 (6.8 – 9.5)
Phosphorus (mg%)	4.2 $\pm$ 0.3 (3.2 – 5)
Alkaline phosphatase (IU/L)	1168 $\pm$ 156 (1002 – 1681)
Alkaline phosphatase in subjects with calcium < 8.6 mg%	1187 $\pm$ 179 (1004 – 1508)

Asian children who had hypocalcemia or normocalcemia, with a high level of ALP.<sup>9-13</sup> The similar abnormal biochemical findings in our study are also supported by other studies from Africa or Bangladesh.<sup>1, 2, 14</sup> After therapeutic intervention with calcium, serum ALP became normal, which is in agreement with a previous study.<sup>15</sup>

We did not determine 24-hour urine calcium in the subjects, because it was very difficult, time consuming, and expensive. Besides, the girls did not cooperate to collect the samples.

Pettifor et al<sup>3</sup> described nine patients with high serum ALP and normal phosphorus. Of them, only two were hypocalcemic, which supports our investigation.

The reason for a low calcium intake may be due to the nutrients such as bread, which can impair intestinal calcium absorption, because of the presence of high amounts of phytic acid in wheat flour.<sup>16</sup> The main source of phytic acid is from bread made from wheat flour.<sup>17</sup> The importance of a 2 – 4-hour leavening process in reducing the phytic acid content of wheat flour had been reported.<sup>18</sup> Furthermore, the widespread consumption of unleavened breads could induce a major public health nutritional problem and it might lead to rickets or osteomalacia in Pakistani and Indian patients,<sup>19</sup> as well as Iranian adolescent girls, suggesting that a low calcium intake may be the main factor.

Three major diagnostic procedures are available for monitoring the bone turn over status and evaluation of metabolic bone disease. They include bone imaging techniques, bone biopsy, and biochemical markers of bone turn over such as ALP. The use of bone density in detecting high bone turn over at the onset stage or monitoring the acute changes in bone is difficult. Bone biopsy is an invasive procedure, but biochemical marker of bone can serve as a routine way to detect and monitor the progression of metabolic bone disease. Thus, the laboratory assessment has been the focus of much attention in recent years. Because the abnormality of bone formation and bone resorption is subtle, the conventional markers such as calcium and parathyroid hormone (PTH) are usually normal.<sup>20, 21</sup>

The current study demonstrated subclinical rickets with one abnormal biochemical parameter, in rapid growth phase of adolescent girls, which calcium deficiency has been the major cause. Prevention of progressive damage and morbidity

rates in adolescent girls depends on the persuasion to change their dietary status.

## Acknowledgments

The authors would like to thank Dr. Asadi, Chief and Manager of Zand Laboratory, Mrs. Maryam Rahnamay-Farzami, and Dr. Fatemeh Rooshan and Dr. Sara Slaleh-Pour for the physical examination of the girls.

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