BIOCHEMICAL FINDINGS IN RICKETS AMONG ADOLESCENT GIRLS

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ABSTRACT

Background: To determine daily calcium, Vitamin D intake and serum biochemical findings of rickets in adolescent girls.

Methods: A total of 414 healthy adolescent student girls aged 11-15 years were evaluated from various areas of Tehran, Iran with different socioeconomic status. A randomized, cross-sectional, prospective and descriptive study was undertaken for calculation of daily calcium, phosphorus intake and vitamin D acquisition by sunlight exposure by seven day recall record questionnaire. Serum 25-hydroxyvitamin D, parathyroid hormone, calcium, phosphorus and alkaline-phosphatase levels were measured.

The serum abnormal biochemical findings of girls were divided as follows: normal or low calcium with raised alkaline phosphatase, group I; normal or low calcium with raised alkaline phosphatase, normal or raised parathyroid hormone, low 25-hydroxyvitamin D, group II; and low phosphorus and 25-hydroxyvitamin D with raised parathyroid hormone, group III.

Results: A total of 44 (10.62%) girls of 414 had abnormal biochemical findings, of these 29 (7%) were in group I, 9 (2.17%) in group II, and 6(1.45%) in group III. The mean daily calcium intake and vitamin D acquisition by sunlight exposure and dietary intake were 360.85±350.50mg and 119.2±52.9 IU respectively. All girls had inadequate dietary calcium and vitamin D intake. All had less than 40 minutes sun exposure per day.

Conclusion: This survey demonstrated that abnormal biochemical findings of rickets can occur even in sunny climates and are caused by two factors, inadequate calcium intake as the major factor and vitamin D deficiency as a minor factor.

Keywords: Rickets, Nutrition, Vitamin D, Calcium, adolescent girl.

INTRODUCTION

In developing countries, rickets due to dietary deficiency of vitamin D usually occurs in the first two years of life. The development of nutritional rickets during rapid growth in puberty has been reported in adolescent Asians and North Africans living in developed countries with a cold climate. This was attributed to low vita-
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min D intake, skin pigmentation, a possible genetic disability to synthesis of cholecalciferol or to convert it to its more active metabolites or increased metabolic demands as a result of rapid growth at puberty and lack of sunlight that is the major source of vitamin D for humans due to the UV-irradiated skin with only a small dietary contribution.2,3

In an epidemiologic study about serum vitamin D levels in the normal and healthy population of Tehran during 2000, it was demonstrated that 47.7% of females aged between 10-19 had severe (<10 ng/mL) or moderate (10-20 ng/mL) vitamin D deficiency.4

The dress code for Iranian Moslem adolescent girls and women is strict and involves total coverage of the body with long garments and a dark maghnaeh (Islamic scarf) for their head and hair. In addition, the middle school student girls can not take off their maghnaeh and long garments even during break and exercise hours.

To our knowledge, all studies are only about symptomatic rickets and suggest that subclinical rickets should be more prevalent than symptomatic rickets. The purpose of this study is to investigate the daily requirement of calcium, phosphorus and vitamin D by dietary intake and sunlight, and the biochemical findings of rickets in a series of healthy Iranian adolescent girls in a sunny and temperate climate environment and discuss risk factors for the development of rickets. Tehran is located at latitude 35°26N, longitude 51°25 E with an altitude of approximately 1200 meters above sea level with a temperate climate.

The study was approved by the research and ethics committee of Shahid Beheshti Medical University.

SUBJECTS AND METHODS

This survey is randomized, cross-sectional, prospective and descriptive from February through March 2003. The study group comprises healthy adolescent guidance school (middle school) girls aged 11-15 years from various areas of Tehran, Iran. All subjects were in a wide spectrum of different socioeconomic status and had no chronic or previous disease that could affect the metabolism or biochemical findings of rickets.

A self-reported questionnaire was used to collect information on the subjects' daily habits. Subjects were requested to report their parental educations and employments, number of family, type of house, ordinary daily food intake, use of calcium or vitamin D supplements, daily use of soft drinks and milk, the average daily duration of sunlight exposure on their face and hands during school term, and holidays, physical activities in regard to hours spent on school exercise and leisure activities. The mean daily dietary intake of calcium, phosphorus and vitamin D were estimated using the seven day recall method. A nutritionist checked each questionnaire and calculated approximate daily intake of calcium, phosphorus, vitamin D and skin sunlight exposure for vitamin D synthesis. Weight, height, and Tanner pubertal stages of all girls were recorded on questionnaires. Physical examination and taking previous history of disease were conducted by two pediatricians. None of the girls showed any signs and symptoms of rickets.

The approximate calcium and vitamin D content of the diets and skin sunlight exposure was calculated by a nutritionist. Fasting blood samples were collected by venipuncture.

After blood centrifugation, serum was separated and stored at −20°C until analysis of 25-hydroxyvitamin D (25[OH] D) and parathyroid hormone (PTH) were measured by radioimmunoassay (Gamma counter system, Genesys). Routine blood chemistry including serum calcium, phosphorus and alkaline phosphatase (ALP) were analyzed by Hitachi 717 system, Autoanalyser RXT Technicon.

The normal serum laboratory reference range of biochemical findings include 25(OH) D (7.6-75 ng/mL), PTH (16-62 pg/mL), ALP (170-1000 Iu/L), calcium (8.6-10.6mg%) and phosphorus (2.5-5 mg%).

Statistical analysis was conducted by SPSS 11.5. Statistical software was used to run the statistical analyses. The results were expressed as mean±standard deviation (SD). The significance level was set at p<0.05. Comparisons of means between groups were done with two-sample t-tests.

The abnormal biochemical findings of rickets were divided as follows: normal or low serum calcium with raised ALP, group I; normal or low serum calcium with

<table>
<thead>
<tr>
<th>Characteristics (units)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>13±1</td>
<td>11-15</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47±11.36</td>
<td>27.5-77.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157±7.65</td>
<td>130-160</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>3.1±1.06</td>
<td>1-5</td>
</tr>
</tbody>
</table>

Table I. Characteristics of cases.

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Table II. Biochemical findings.

<table>
<thead>
<tr>
<th>Biochemical findings</th>
<th>Cases</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within normal range</td>
<td>370</td>
<td>89.38</td>
</tr>
<tr>
<td>Group I</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>Group II</td>
<td>9</td>
<td>2.17</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>1.45</td>
</tr>
<tr>
<td>Sum</td>
<td>414</td>
<td>100</td>
</tr>
</tbody>
</table>

Table III. Results of serum biochemical findings in girls.

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>P</th>
<th>ALP</th>
<th>PTH</th>
<th>25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>9.45±0.536</td>
<td>4±0.657</td>
<td>613±307.2</td>
<td>41.61±24.4</td>
<td>30.123±15.75</td>
</tr>
<tr>
<td>Range</td>
<td>6.6-10.9</td>
<td>2-8</td>
<td>125-1964</td>
<td>14.9-277</td>
<td>5.1-71.2</td>
</tr>
<tr>
<td>Group I</td>
<td>9.33±0.39</td>
<td>—</td>
<td>1168.4±156.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Range</td>
<td>8.2-9.5</td>
<td>—</td>
<td>1002-1681</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group II</td>
<td>8.025±1.04</td>
<td>—</td>
<td>1450.9±357.2</td>
<td>144.87±74.5</td>
<td>6.76±0.95</td>
</tr>
<tr>
<td>Range</td>
<td>6.8-9.4</td>
<td>—</td>
<td>1009-1964</td>
<td>65-277</td>
<td>5.1-8</td>
</tr>
<tr>
<td>Group III</td>
<td>—</td>
<td>2.4±0.352</td>
<td>—</td>
<td>78.83±23.06</td>
<td>8.98±2.05</td>
</tr>
<tr>
<td>Range</td>
<td>—</td>
<td>2-2.9</td>
<td>—</td>
<td>60-119</td>
<td>6.9-12</td>
</tr>
</tbody>
</table>

Ca: calcium, P: phosphorus
Units of parameters are shown in mg%/±SD for Ca and P, IU/L±SD for ALP, pg/mL for PTH and ng/mL±SD for 25-(OH)D.

Table IV. The dietary intake, exercise and daily sunlight results.

<table>
<thead>
<tr>
<th>Variable (units)</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D intake (IU/d)</td>
<td>119.2±52.91</td>
<td>12.5-312.5</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>360.85±350.50</td>
<td>33-2623</td>
</tr>
<tr>
<td>Phosphorus intake (mg/d)</td>
<td>1137.84±550.814</td>
<td>108-4500</td>
</tr>
<tr>
<td>Exercise (min/d)</td>
<td>10</td>
<td>10-30</td>
</tr>
<tr>
<td>Sunlight exposure (min/d)</td>
<td>10</td>
<td>5-40</td>
</tr>
</tbody>
</table>

Raised ALP, low 25 (OH)D, normal or raised PTH concentration, group II; and low phosphorus and 25(OH)D with raised PTH concentration, group III. Subjects with renal or hepatic disease, malabsorption or anticonvulsant therapy were excluded.

RESULTS

A total of 414 adolescent girls were evaluated for biochemical findings of rickets. The characteristics of the girls are shown in Table I.

All were between Tanner pubertal stages 1-5 with no significant differences in weight and height between total normal biochemical findings of girls and the three abnormal groups. The only significant differences were Tanner stages between normal and abnormal groups (p<0.05).

The overall results of the three groups are shown in Table II.

A total of 12 girls were hypocalcemic, of these 8 were in group I and 4 in group II. Serum ALP was increased in 38 girls, of these, 29 were in group I and 9 in group II. Serum 25 (OH) D was reduced in 15 girls, 9 in group II and 6 in group III. Serum PTH was increased in 15 girls, 9 in group II and 6 in group III.

The results of biochemical findings of the three groups are shown in Table III.

The dietary intake, exercise and daily sunlight expo-
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Table V. Daily calcium and vitamin D intake in the three groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ca (Mean±SD)</th>
<th>Vitamin D (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>585.5±265.82</td>
<td>119±34.41</td>
</tr>
<tr>
<td>Ranges</td>
<td>274-1010</td>
<td>100-185</td>
</tr>
<tr>
<td>II</td>
<td>471.58±383.98</td>
<td>110±59.76</td>
</tr>
<tr>
<td>Ranges</td>
<td>72-1447</td>
<td>0-240</td>
</tr>
<tr>
<td>III</td>
<td>621±290.83</td>
<td>123.92±45.14</td>
</tr>
<tr>
<td>Ranges</td>
<td>206-1381</td>
<td>0-200</td>
</tr>
</tbody>
</table>

Ca in mg%, vitamin D in International units.

sure values in all of the cases are shown in Table IV.

Dietary evaluation revealed that bread is the main source and then cereal, rice, meat, vegetables, milk and milk products are not as important a source of dietary calcium intake. The milk is not fortified with vitamin D, and is consumed infrequently and with low amounts. Nobody was a vegetarian. Approximately 32(8%) received a daily dietary calcium intake above the 1200 mg recommended daily allowance for all age groups; of these, 3 had low vitamin D, 1 low serum calcium, and 3 raised alkaline phosphatase. None was receiving vitamin D and calcium supplementation. There were no significant differences between daily calcium intake of normal and total abnormal findings of groups. All had a daily vitamin D intake below the recommended daily allowance of 400 IU. The daily intake of calcium and vitamin D in the three groups are shown in Table V.

The mean daily sun exposure was 10 minutes (range 5-40 minutes). Exposure for less than 40 minutes was reported in all girls.

**DISCUSSION**

Nutritional rickets may be caused by inadequate vitamin D or calcium intake, especially during rapid growth phases. Vitamin D is essential for the maintenance of calcium homeostasis and bone mineralization. Under normal conditions the endogenous synthesis of vitamin D is more important than that obtained by dietary intake. Our data revealed that low vitamin D in adolescent girls consists in two groups. As we previously showed, group II and III were associated with low serum vitamin D concentrations; this may be due to the skin pigmentation (some of the population), low skin exposure to UV radiation (all of the population), season, air pollution and lifestyle. Hypovitaminosis D may be associated with decreased calcium absorption and failure of the osteoid to mineralize, and this may ultimately lead to overt clinical rickets. However no definitive level can be given below which rickets occurs, but children with vitamin D deficiency rickets usually have low or undetectable 25-(OH) D levels. As a result of vitamin D deficiency, calcium absorption will be reduced and the PTH level will rise to maintain plasma calcium at a physiologically optimum level by inducing calcium mobilization from the bones. As a result, secondary hyperparathyroidism will occur. This study demonstrated secondary hyperparathyroidism in all girls in group III and 7 of 9 girls in group II. In this study, 15 girls (3.62%) are vitamin D deficient, despite the results of another study in Tehran. However, numerous studies demonstrated symptomatic rickets in African and Asian children with hypocalcemia or normocalcemia with high alkaline phosphatase levels. Similar abnormal biochemical findings in this study consist in group I (7% the largest group) and supported other studies from Africa or Bangladesh. The consumption of calcium and serum calcium concentrations in girls with normal or abnormal findings is approximately similar, but plasma ALP activity consistently is higher in girls with abnormal biochemical findings. The daily calcium intake in group I girls is low: mean 587.5±265.82 mg% and phosphorus high. In addition, the main constituent of their foods is bread which can impair intestinal calcium absorption because of high phytic-acid in wheat flour. The main source of phytic-acid is from bread made with whole meal wheat flour. The importance of a 2-4 hours leavening process in reducing the phytic-acid content of whole meal wheat flour has been reported and the widespread consumption of unleavened breads induces a major public-health nutritional problem and in Pakistani, Indian patients may be important in causing rickets or osteomalacia. In regard to African patients with insufficient dietary calcium who develop rickets, our adolescent girls in group I suggest that low calcium intake was the main factor.

The serum concentration of 25(OH) D is the most sensitive biochemical marker of a subject’s vitamin sta-
The present study demonstrated a frequency of hypovitaminosis D in 15 girls (3.62%). The mean daily acquired vitamin D in all girls was approximately less than 100 IU/d whereas the remainder of girls had no abnormal biochemical findings. The explanation of these differences may be due to incorrect information writing in the questionnaire by students or eating habits between the two groups. In the current study the mean 25(OH)D concentration in the two groups were low (6.76 ng/mL) whereas in healthy 9-15 year-old Finnish girls this was 13 ng/mL (33.9 nmol/L). The total mean vitamin D acquisition by dietary intake and sunlight exposure in girls was 119.2 IU/d that is below the recommended daily allowance. Assessment of dietary intake and sun exposure by history alone has limitation of accuracy. However it was the best method available for this study because home and school visits to assess dietary intake or sun exposure would have been culturally unacceptable.

The association between iron deficiency and vitamin D deficiency has been described in the UK in Asian preschool children. This may be because dietary and lifestyle factors such as rapid growth and onset of menstruation lead to deficiency in both nutrients of our study girls. The beneficial effects of iron supplementation on vitamin D status described and suggest that iron deficiency affects gut absorption of vitamin D. However, we did not measure serum iron or hemoglobin levels in the girls but as we have shown it can be due to lifestyle and nutrient factors. The other factor with contributed to vitamin D deficiency is unfortified milk or other foods that these girls received with little or no Vit D. The other main dietary factor which has been associated with vitamin D deficiency is bread. It has been postulated that the high phytate content of bread may interfere with the enteroh epatic circulation of vitamin D metabolites. In one adult study, high phytate consumption accounted for 12% of the variance in vitamin D levels and was a significant risk factor for rickets in the UK. McKenna reported that the mean vitamin D intake is significantly lower in central Europe than in Northern America that is compatible with the two groups of the current study. Utiger suggested that vitamin D supplementation should be used more widely, and also peripubertal children should consume daily vitamin D supplementation. A low exposure to sunlight and reduced synthesis of vitamin D has also been implicated in rickets. In the present study the daily sunlight exposure in abnormal biochemical findings of girls was inadequate.

Whether this is transient or persistent and will show their symptoms in the future is unknown and requires further investigations and follow-up that are now impossible in our situation because of abundant limita-

tions and problems.

**CONCLUSION**

The current study is a screening investigation that demonstrates abnormal biochemical findings of rickets in adolescent girls with mixed etiology. The majority of girls were calcium deficient and the remainder vitamin D deficient. It seems that prevention of rickets in adolescent girls of our country depends on persuading them to change their eating habits and a program of dietary education should be suggested by the ministry of health. Sunlight exposure should be increased by sequential programs so as to prevent progressive damage and the high morbidity rates of rickets in adolescent girls.

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