The Turkish Journal of Pediatrics 29:155 - 162, 1987

SERUM - 25 - HYDROXYCHOLECALCIFEROL LEVELS IN CHILDREN AND ADOLESCENTS*

*Asım Gültekin MD***, İmran Özalp MD****, Alev Hasanoğlu MD*****
*Ahmet Ünal PhD******

Key words: serum-25-hydroxycholecalciferol, vitamin-D deficiency, children, adolescence

Nutritional rickets is still one of the important health problems of developing countries[1-5]. In Turkey, the rate of rickets is high and has been reported to be 19% in the slums of Istanbul and 6.36% in Sivas[5-7].

Clinical osteomalacia and rickets are very common disorders among Asian immigrants in the United Kingdom[8]. In this group, the overall incidence of clinical disease has been reported to be about ten percent, while biochemical disease twenty-five percent over all ages or fifty percent in adolescents alone[9-13].

Since no correlation could be found between the occurrence of rickets and the Asian children's residing in Britain, it was suggested that the vitamin D depots of these children were not sufficient, even when they were in their own sunny countries. Their vitamin D deficient diet consisted mainly of a type of unleavened bread called chapattis, which played an important role in their high percentage of vitamin-D deficiency[14,15].

In school-age children, especially in adolescents, there is a greater need for vitamin D due to rapid development. When these children lack a sufficient vitamin D intake and exposure to sunlight, subclinical rickets may develop[8,16,17].

Since it had been observed in an earlier study on adolescent pregnancies that the serum-25-hydroxycholecalciferol (serum-25-HCC) levels were rather low in Ankara[18], attempts were made to determine these levels in school children, especially in adolescent girls in Sivas.

* From the Department of Pediatrics, Cumhuriyet University Faculty of Medicine, Sivas.
** Associate Professor of Pediatrics, Cumhuriyet University Faculty of Medicine.
*** Professor of Pediatrics, Hacettepe University Institute of Child Health, Ankara.
**** Associate Professor of Pediatrics, Erciyes University Faculty of Medicine, Kayseri.
***** Assistant Professor of Physiology, Cumhuriyet University Faculty of Medicine.

Material and Methods

This study was carried out on ninety subjects selected at random from amongst students attending elementary, secondary or high school in Sivas. Eight to eleven subjects were chosen from each class. The approximate daily vitamin D and calcium intake was calculated by a one week diet history. The social and economic conditions of the families were established by inquiring as to their monthly income, family size and the educational level of the parents. The subjects were divided into two groups according to the replies given.

Group I comprised 24 girls and 20 boys whose ages ranged between 6-17 years. Their diet, which mainly consisted of vegetable source foods also included "bazlama" (a type of unleavened bread similar to chapattis), and very little animal source protein. There were from three to eight siblings in each subject's family. Eighteen mothers were totally illiterate, 20 only had attended primary school, and six secondary school. Twenty-five fathers had attended primary school, 16 secondary school and three the university.

Group II comprised 17 girls and 29 boys whose ages ranged between 6-17 years. Their diet, which was more nutritious than that of Group I, consisted mainly of animal source protein and leavened bread. There were from two to six siblings in each subject's family. Eight mothers were totally illiterate, 14 had attended primary school, 12 secondary school, and 12 high school. Sixteen fathers had attended primary school, 20 secondary school and 10 high school.

In order to prevent seasonal variations, blood samples were taken between 15 February - 15 March. The sera were separated within two hours and stored at −20 °C. Serum calcium was estimated by the Ferro and Ham[19], serum phosphate by a modified Goldenberg[20], alkaline phosphatase by a modified Bodensky[21], and total protein and albumin by the Biuret[22] methods. Serum magnesium was determined by the Perkin Elmer-Model 103 atomic absorption spectrophotometer[23], and the serum-25-HCC levels were estimated according to the method described by Taylor et al[24,25] (levels below 4 ng/ml considered vitamin D deficiency).

Results

Group I: The average daily intake of calcium and vitamin D of these children was 433.86 ± 125.16 mg and 45.25 ± 10.78 i.u. respectively. The ranges of the biochemical evaluations of the sera were: serum calcium 9-11 mg/dl, phosphate 3.5 - 4.8 mg/dl, alkaline phosphatase 5.5 - 9.2 BU, total protein 5-7 g/dl, albumin 3.3 - 4.5 g/dl, magnesium 1.5 - 2.8 mg/dl, and serum-25-HCC 2.05 - 17.70 ng/ml. In seven cases, the serum-25-HCC level was 4 ng/ml or 15.9% (Figs. 1, 2).

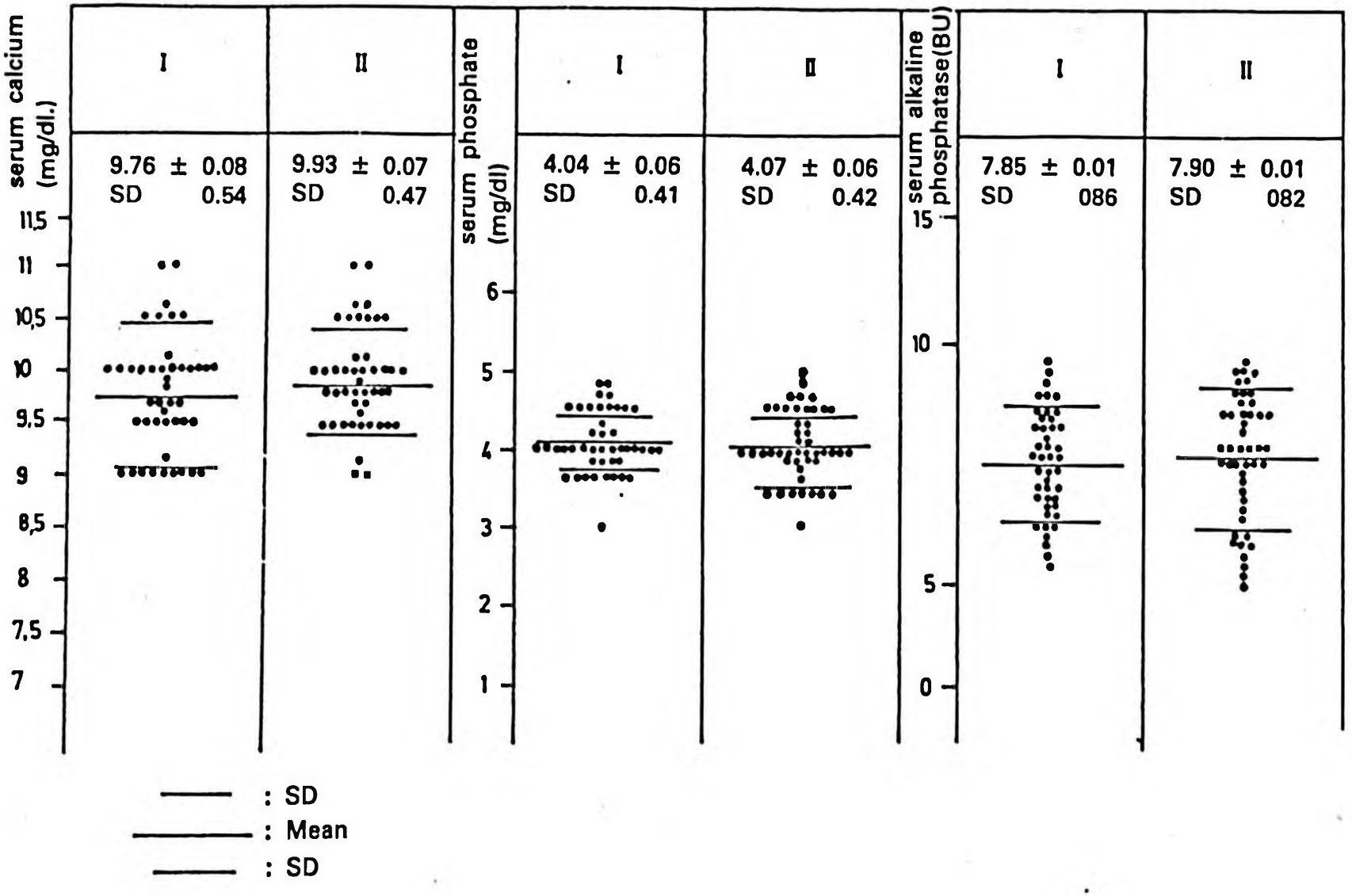

Fig. 1: The distribution of serum calcium, phosphate, alkaline phosphatase levels in Groups I and II.

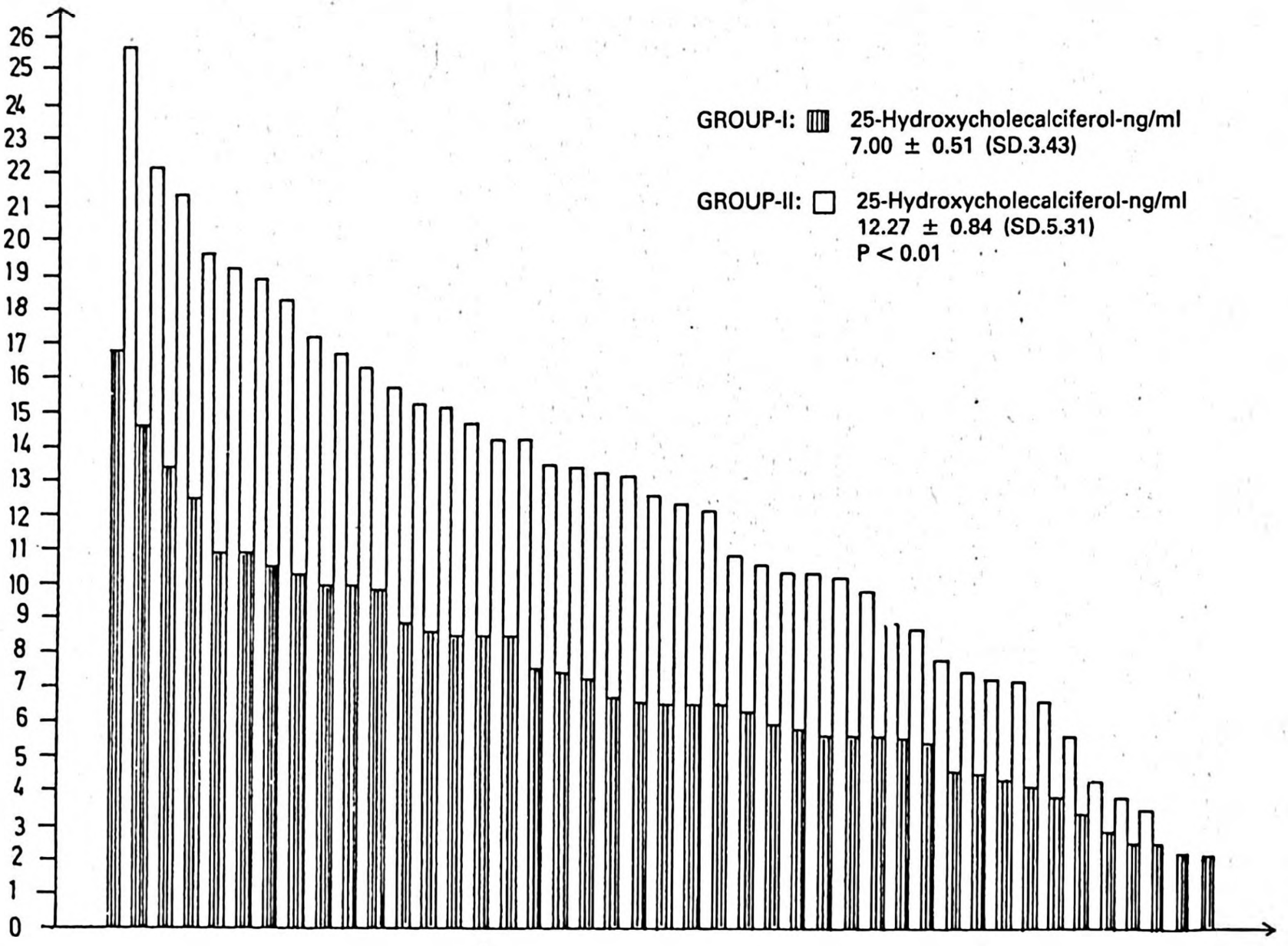

Fig. 2: Serum-25-Hydroxycholecalciferol levels in Groups I and II.

Group II: The average daily intake of calcium and vitamin D of these children was 829.78 ± 193.20 mg and 78.56 ± 17.88 I.U. respectively. The ranges of the biochemical evaluations of the sera were: serum calcium 9-11 mg/dl, phosphate 3.5 - 4 mg/dl, alkaline phosphatase 8-9.5 BU, total protein 5.6 - 7.6 g/dl, albumin 3.5 - 4.8 g/dl, magnesium 1.5 - 2 mg/dl, and serum-25-HCC 3.30 - 22.10 ng/ml (in six cases the serum-25-HCC levels could not be determined).

When the serum laboratory values of both groups were compared, the total protein, albumin and serum-25-HCC values of the second group were found to be statistically significant ($P < 0.01$; Table I, Figs. 1, 2).

The children of both groups were divided into two groups according to age (6-11 and 12-17 year-old age-groups) and sex. It was observed that the serum-25-HCC values decreased in the older subjects of both sexes. When these values were compared, this decrease was found to be statistically significant (Table II).

TABLE I: The Comparison of Serum Laboratory Values in Groups I and II.

		GROUP I				GROUP II					
		n	xn	SD	SX	n	$\bar{x}$	SD	SX	t	p
Mg	(mg/dl)	44	2.06	0.38	0.05	43	1.92	0.35	0.05	1.76	> 0.05
Ca	(mg/dl)	44	9.76	0.54	0.08	46	9.93	0.47	0.07	1.53	> 0.05
P	(mg/dl)	44	4.04	0.41	0.06	46	4.07	0.42	0.06	0.34	> 0.05
AP	(B.U)	44	7.85	0.86	0.13	46	7.90	0.82	0.01	0.29	> 0.05
T.P	(g/dl)	44	6.16	0.37	0.05	46	6.55	0.50	0.07	4.17	< 0.01
Alb	(g/dl)	44	3.76	0.33	0.04	46	4.05	0.34	0.05	4.10	< 0.01
25-HCC	(ng/ml)	44	7.00	3.43	0.51	40	12.27	5.31	0.84	5.32	< 0.01

Magnesium (Mg). Calcium (Ca). Phosphate (P). Alkaline phosphatase (AP). Total protein (T.P). Albumin (Alb). 25-Hydroxycholecalciferol (25-HCC).

TABLE II: The Comparison of Serum-25-HCC Levels in Groups I and II According to Age and Sex.

		GROUP I			GROUP II				
sex	age	n	$\bar{x}$	Sx	n	$\bar{x}$	Sx	t	p
25-HCC girls	6-11	16	7.23	0.43	8	15.65	1.67	3.70	< 0.01
	12-17	8	3.39	0.55	9	5.82	0.72	2.66	< 0.02
25-HCC boys	6-11	8	10.13	1.68	11	15.60	1.44	2.47	< 0.02
	12-17	12	7.03	0.88	12	13.12	1.09	4.33	< 0.01

Discussion

As is known, serum-25-hydroxycholecalciferol levels which are the most important vitamin D metabolites, determine the state of vitamin D in the organism[26]. It is also known that the level of serum-25-HCC varies according to geography, social and economic conditions, eating habits, daily vitamin D intake and exposure to sunshine[27,28]. In a study carried out in England, it was established that the average serum-25-HCC levels in healthy people were 12.1 ± 0.8 ng/ml, whereas in the Asians, who had settled in that country, it was 7.2 ± 1.2 ng/ml[29].

It has been reported that the average serum-25-HCC level was 25 ng/ml in the United States. The difference between the levels of these countries seems to indicate that people in the United States prefer foods rich in Vitamin D and also expose themselves to more sunlight than those living in England. Similar results have also been obtained in an investigation carried out in Johannesburg[30].

In our country, serum 25-hydroxycholecalciferol levels have also been determined in pregnant women and in newborn infants, and in winter, owing to seasonal changes, their average serum-25-HCC levels were found to be 5.22 ± 0.97 ng/ml and 2.60 ± 0.73 ng/ml, respectively. On the other hand, when these two groups were examined in summer, the serum-25-HCC levels were found to be 14.32 ± 1.54 ng/ml and 4.91 ± 1.22 ng/ml, respectively. These levels were found to be lower particularly in the offspring of adolescent pregnancies[31].

Since vitamin D intake in school-age children, especially adolescents, should be higher for developmental reasons, any lack in this may likely result in subclinical rickets[8,16,17]. It is a known fact that with a view to establishing serum vitamin D levels and subclinical rickets, various research has been carried out[8,14,15,17,19,31].

Studies carried out on Asian children residing in England showed that the rate of subclinical rickets in these children could reach fifty percent at puberty[8-13]. Deficient vitamin D intake, insufficient exposure to sunlight as well as a diet containing chapattis were regarded as being responsible for this high rate of rickets[14,15].

We found in our research that the average serum-25-HCC level for Group I was 7.00 ± 0.51 ng/ml and for Group II, 12.27 ± 0.84 ng/ml. The difference between the two groups was found to be statistically significant ($p < 0.01$). Although, the daily intake of vitamin D and the nutritional conditions of Group II were the factors which most likely caused this difference, we also believe that the diet followed by Group I, which contained "bazlama", may be another factor responsible for this difference. When the ages of these subjects and the serum-25-HCC levels were compared, it was observed that these levels decreased in the adolescents of both

groups, and that this decrease was more obvious in the girls than in the boys ($p < 0.001$ and $p < 0.05$, respectively; Table II).

Serum calcium, phosphate and alkaline phosphatase levels do not generally suffice in diagnosing subclinical rickets[16,17]. For this reason in asymptomatic cases with normal serum calcium, phosphate and alkaline phosphatase levels, the determination of a low level of serum-25-HCC shows that 25-hydroxycholecalciferol assay is a very sensitive method in diagnosing subclinical rickets. Our findings have led us to believe that vitamin-D deficiency in our country is an important problem not only in early childhood but in school-age children as well.

The determination of low serum 25-HCC levels, particularly in adolescent females, indicates that the offspring resulting from adolescent marriages, frequently encountered in our country, may likely suffer from vitamin-D deficiency.

Summary

This study was carried out to determine the relation between the nutritional status and serum-25-hydroxycholecalciferol levels in school age children. In Group I, whose diet maily consisted of vegetable source foods, vitamin D deficiency was recorded as 15.9 percent while in Group I, whose nutrition was more balanced, this figure was 5 percent. This finding led us to believe that vitamin D deficiency in our country is an important problem not only in infancy but also in school-age children, especially in adolescent girls.

REFERENCES

1. American Academy of Pediatrics Report of the Committee on Nutrition. Infantile Scurvey and nutritional rickets in the United States. *Pediatrics* 28:646, 1962.
2. Salimpour R. Rickets in Tehran. Study of 200 cases. *Arch Dis Child* 50:63, 1975.
3. Edidin DV, Levitsky LL, Schey W, et al. Resurgence of nutritional rickets associated with breast-feeding and special dietary practices. *Pediatrics* 65:232, 1980.
4. Ocal G, Berki R, Abal G, ve ark. Süt çocukluğu döneminde güncelliğini koruyan D vitamini yetersizliğine bağlı raşitizm sorunu. *Çocuk Sağlığı ve Hastalıkları Dergisi* 26:39, 1983.
5. Tümay SB, Bilger M. D vitamini ile ilgili rahitis (noksanlık rahitisi) çocuklarda kalsiyum, fosfor metabolizması ve malnütrisyon. XI. Türk Pediatri Kongresi. 17-22 Temmuz 1972, Istanbul: Sermet Matbaası, 1974, s. 66.
6. Gültekin A, Savaş A, Özalp İ. Nütrisyonel raşitizmin 150,000 İ.Ü. D_3 vitamini ile tedavisinın sonuçları. *Çocuk Sağlığı ve Hastalıkları Dergisi* 27:169, 1984.
7. Gültekin A, Savaş A, Özalp İ. 0-3 yaş grubunda raşitizmin görülme sıklığı. *Çocuk Sağlığı ve Hastalıkları Dergisi* 28:119, 1985.
8. Hunt SP, O'Riordan JL, Windo J, et al. Vitamin D status in different subgroups of British Asians. *Br Med J* 2:1351, 1976.

9. Goel KM, Sweet EM, Logan RW, et al. Florid and subclinical rickets among immigrant children in Glasgow. *Lancet* 1:1141, 1976.
10. Cooke WT, Swan CH, Asquith P, et al. Serum alkaline phosphatase and rickets in urban school children. *Br Med J* 1:324, 1973.
11. Monriceff MW, Lunt HR, Arthur LJ. Nutritional rickets at puberty. *Arch Dis Child* 48:221, 1973.
12. Ellis G, Woodhead JS, Cooke WT. Serum-25-hydroxyvitamin D concentrations in adolescent boys. *Lancet* 1:825, 1977.
13. Ford JA, McIntosh WB, Butterfield R,et al. Clinical and subclinical vitamin D deficiency in Bradford children. *Arch Dis Child* 51:939, 1976.
14. Holmes AM, Enoch BA, Taylor JL, Jones ME. Occult rickets and osteomalacia amongst the Asian immigrant population. *Q J Med* 42:125, 1973.
15. Dunnigan MG, McIntosh WB, Ford JA. Rickets in Asian immigrants *Lancet* [letter] 1:1346, 1976.
16. Pettifor JM, Isdale JM. Diagnosis of subclinical rickets. *Arch Dis Child* 55:155, 1980.
17. O'Hare AE, Uttley WS, Belton NR, et al. Persisting vitamin D deficiency in the Asian adolescent. *Arch Dis Child* 59:766, 1984.
18. Hasanoğlu A, Özalp İ, Özsoylu Ş. Anne ve kondon kanında serum 25-hidroksikolekalsiferol değerleri. *Çocuk Sağlığı ve Hastalıkları Dergisi* 24:207, 1981.
19. Ferro PV, Ham AB. A simple spectrophotometric method for the determination of calcium. *Am J Clin Pathol* 28:208, 1957.
20. Goldenberg H, Fernandoz A. Simpilified method for the estimation of inorganic phosphorus in body fluids. *Clin Chem* 12:871, 1966.
21. Gradwohl RB. Alkaline phosphatase Bodansky (modified). In Frankel S, Reitman S, Sonnenwirth AC (eds). *Gradwohl's Clinical Laboratory Methods and Diagnosis: A Textbook on Laboratory Procedures and their Interpretation* (7th ed). Saint Louis: Mosby Co, 1970, p 50.
22. Fister HJ. *Manual of Standardized Procedures for Spectrophotometric Chemistry.* New York: Standard Scientific Supply, 1950, p 50.
23. Hansen JL, Freier EF. The measurement of serum magnesium by atomic absorption spectrophotometry. *Am J Med Tech* 33:158, 1967.
24. Haddad JG, Chyu K. Competitive protein-binding radioassay for 25-hydroxycholecalciferol. *J Clin Endocrinol Metab.* 33:992, 1971.
25. Taylor CM, Hughes SE, DeSilva P. Competitive protein-binding assay for 24,25-dihydroxycholecalciferol. *Biochem Biophys Res Commun* 70:1243, 1976.
26. Gerol AD, Stephan JM, Allen MS. Metabolic bone disease. In Williams RH (ed). *Textbook of Endocrinology* (7th ed). Philadelphia: WB Saunders Co, 1985 p 1218.
27. Specker BL, Valanis B, Hertzberg V, et al. Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infants. *J Pediatr* 107:372, 1985.
28. Juttmann JR, Visser TJ, Buurman C, et al. Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects. *Br Med J* 282:1349, 1981.
29. Preece MA, Tomlinson S, Ribot CA, et al. Studies of vitamin D deficiency in man *Q J Med* 44:575, 1975.
30. Pettifor JM, Ross FP, Moodley G, et al. Serum calcium, magnesium, phosphorus, alkaline phosphatase and 25-hydroxyvitamin D concentrations in children. *S Afr Med J* 53:751, 1978.
31. Hasanoğlu A, Özalp İ, Özsoylu Ş. Yenidoğan döneminde serum 25-hidroksikolekalsiferol değerlerinin anne ve kondon kanı değerleri ile karşılaştırılması. *Çocuk Sağlığı ve Hastalıkları Dergisi* 24:215, 1981.