Evidence of Hypovitaminosis D in Patients with Mitral Ring Calcification

Masataka SHIRAKI, M.D.,* Akihiko MIYAGAWA, M.D.,**

Itaru AKIGUCHI, M.D.,*** Hideki ITO, M.D.,***

Shin-ichiro OHKAWA, M.D.,** and Masaya SUGIURA, M.D.**

SUMMARY

In order to evaluate the role of calcium regulating hormones in the pathogenesis of mitral ring calcification, we have studied the serum levels of PTH and vitamin D metabolites in aged females both with and without mitral ring calcification (MRC).

In the patients with MRC ($n=17$), significantly lower levels of serum total protein (6.6 ± 0.2 in the MRC group vs 7.1 ± 0.1 g/dl in the control group, mean $\pm$ SEM), BUN (15.7 ± 0.9 vs 18.3 ± 0.9 mg/dl), creatinine (0.7 ± 0.02 vs 0.9 ± 0.02 mg/dl) and calcium (8.4 ± 0.1 vs 9.2 ± 0.1 mg/ml) were observed as compared with those in the controls ($n=32$). Significantly higher PTH levels (0.57 ± 0.07 vs 0.38 ± 0.04 ng/ml) were found in the MRC group. Levels of all three vitamin D metabolites in the MRC group were significantly lower than those in the control group (25-OHD; 11.2 ± 1.4 vs 19.6 ± 1.2 ng/ml, 24,25(OH) $_2$ D; 0.7 ± 0.1 vs 1.3 ± 0.1 ng/ml and 1,25(OH) $_2$ D; 12.5 ± 2.4 vs 43.0 ± 3.5 pg/ml). The correlation coefficient between PTH and 1,25(OH) $_2$ D was -0.382 ($n=49$, $p<0.01$). Thus, the significantly higher PTH levels in the MRC group might result in hypovitaminosis D.

In conclusion, evidence of hypovitaminosis D in the patients with mitral ring calcification was demonstrated.

Additional Indexing Words:

Vitamin D Mitral ring calcification PTH Aged Secondary hyperparathyroidism Calcium metabolism

IT has long been known that mitral ring calcification (MRC) is frequently observed in aged patients,¹⁾⁻³⁾ especially in women. As suggested by a higher frequency of osteoporosis in aged females than in aged males, abnormalities in calcium metabolism exist more frequently in females than in males. The regulation of calcium metabolism depends primarily on the actions of

From the Section of Laboratory Medicine, * Division of Cardiology, ** and Endocrinology and Metabolism Section, *** Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.

Address for reprints: Masataka Shiraki, M.D., Laboratory of Medicine, Tokyo Metropolitan Geriatric Hospital, 35-2, Sakaecho, Itabashi-ku, Tokyo 173, Japan.

Received for publication October 12, 1987.

Accepted April 13, 1988.

parathyroid hormone (PTH) and vitamin D metabolites on the intestine, kidneys and bones. Therefore, abnormalities of these target organs for calcium regulating hormones result in systemic abnormalities in calcium metabolism. In fact, it has been reported that mitral ring calcification was clearly identified in renal failure which is usually associated with secondary hyperparathyroidism.^{4),5)} Thus, it is interesting to measure serum levels of PTH and vitamin D metabolites in the aged female with or without mitral ring calcification.

SUBJECTS AND METHODS

Subjects:

Forty nine aged female patients were studied. They were divided into 2 groups depending on the presence or absence of mitral ring calcification. The diagnosis of mitral ring calcification was made by M-mode echocardiography in accordance with the criteria reported by Okimoto et al⁶⁾ and Hirschfeld et al.⁷⁾ The MRC group consisted of 17 patients with mitral ring calcification and the control group of 32 patients without mitral ring calcification.

The mean ages of the MRC group and the control group were 80.8 years (range: 65–91) and 75.9 years (range: 64–88), respectively.

Patients with hepatic dysfunction, renal failure and primary hyperparathyroidism were excluded as subjects of the study. All of the patients studied were receiving calcium or vitamin D supplements. The incidence of treated hypertension in the MRC and control groups was 64.7% (11 out of 17 cases) and 53.1% (17 out of 32 cases), respectively.

Methods:

All subjects were hospitalized and received a formulated diet which contained 12 g of sodium chloride, 600 mg of calcium and 1200 mg of phosphate, daily. After allowing 10 days for adaptation to this diet, urine samples were collected for 3 consecutive days for the measurement of urinary calcium (Ca) and inorganic phosphorus (Pi) excretion. The average of these three determinations was used as the value for the each subject. On the last of the 3 consecutive days, blood was drawn from each subject to determine the levels of serum total protein (T.P.), blood urea nitrogen (BUN), serum creatinine, Ca, Pi, PTH, 25-hydroxycholecalciferol (25D), 24,25-dihydroxycholecalciferol (24,25D) and 1,25-dihydroxycholecalciferol (1,25D).

Serum levels of BUN, Ca, Pi, creatinine and T.P. were determined using autoanalyzers (JCAH-120; Nihondenshi, Tokyo and Hitachi 706D, Hitachi, Tokyo, Japan). Serum levels of PTH were analyzed using the Eiken PTH

RIA kit (Eiken Co., Tokyo, Japan) which mainly detects C-terminal fragments of the PTH molecule.⁸⁾ The lowest detectable level of this assay system was 0.1 ng/ml and cv at 1.1 ng/ml of standard serum was 12.5% (n=22). The normal range of this assay in elderly females was 0.16–0.55 ng/ml. Serum levels of vitamin D metabolites were measured by competitive protein binding assay for 24,25D and 25D and by radioreceptor assay for 1,25D after extraction and purification of the samples using column chromatography, as reported previously.⁹⁾ The normal ranges of our assay system for vitamin D metabolites are 13–39 ng/ml for 25D, 1.0–3.3 ng/ml for 24,25D and 11–55 pg/ml for 1,25D.

Data are presented as mean $\pm$ SEM and statistical analysis was performed by unpaired Student's t-test (two-tailed).

RESULTS

1) Background data of the subjects

The background data of the MRC and control groups are summarized in Table I. T.P. ($p < 0.05$), BUN ($p < 0.05$), serum creatinine ($p < 0.001$) and serum calcium ($p < 0.001$) in the MRC group were significantly lower than those in the controls. The urinary Ca/creatinine and Pi/creatinine ratios in the control group were not significantly different from those in the MRC group. The parameters in both groups were within the normal ranges of our hospital.

Table I. Background Data of the Subjects

Parameters	Control (range)	MRC (range)	Significance
Age (y.o.)	75.9 $\pm$ 1.1 (64–88)	80.8 $\pm$ 1.7 (65–91)	n.s.
Total protein (g/dl)	7.1 $\pm$ 0.1 (6.8–8.5)	6.6 $\pm$ 0.2 (5.9–8.0)	$p < 0.05$
BUN (mg/dl)	18.3 $\pm$ 0.9 (7–30)	15.7 $\pm$ 0.9 (8–21)	$p < 0.05$
Creatinine (mg/dl)	0.9 $\pm$ 0.02 (0.5–1.2)	0.7 $\pm$ 0.02 (0.5–0.9)	$p < 0.001$
Calcium (mg/dl)	9.2 $\pm$ 0.1 (8.4–10)	8.4 $\pm$ 0.1 (8–9.1)	$p < 0.001$
Phosphate (mg/dl)	3.4 $\pm$ 0.1 (2.7–4.5)	3.4 $\pm$ 0.1 (2.5–4.2)	n.s.
U-Ca/Cr	0.13 $\pm$ 0.02 (0.02–0.43)	0.14 $\pm$ 0.08 (0.03–0.33)	n.s.
U-Pi/Cr	0.53 $\pm$ 0.04 (0.19–0.89)	0.53 $\pm$ 0.06 (0.18–1.01)	n.s.
Number of cases	32	17	

Urinary calcium and inorganic phosphate concentrations were measured after adaptation to a regular hospital diet containing 12 g of NaCl, 600 mg of calcium and 1200 mg of phosphate daily. Other serum parameters were measured simultaneously at the stage when the urinary samples were obtained. Data are expressed as the mean $\pm$ SEM. The statistical analysis was performed with Student's t-test (two-tailed).

2) Serum levels of calcium regulating hormones

As shown in Fig. 1, the mean serum PTH levels in the patients with mitral ring calcification (MRC group) (0.57 ± 0.07 ng/ml) were significantly higher than those in the control group (0.38 ± 0.04 ng/ml). The serum level of PTH in 1 of the 17 cases in the MRC group exceeded 1 ng/ml and was in the range of primary hyperparathyroidism. However, repeated measurement of her serum Ca and Pi was within the normal range and no other obvious evidence of the presence of a parathyroid tumor was detected. All the serum levels of vitamin D metabolites (25D; 11.2 ± 1.4 ng/ml, 24,25D; 0.7 ± 0.1 ng/ml and 1,25D; 12.5 ± 2.4 pg/ml) in the MRC group were significantly lower than those in the control group (19.6 ± 1.2 ng/ml, 1.3 ± 0.1 ng/ml and 43.0 ± 3.5 pg/ml, respectively) (Fig. 2). Serum 25D levels in 12 out of 17 patients in the MRC group were below the normal range for their age. Thirteen of the 17 cases with mitral ring calcification had levels of 24,25D and 10 cases had levels of 1,25D which were under the lower limits of the normal range for the aged. In contrast, 7 of the 32 controls had levels of 25D and 13 cases had levels of 24,25D which were under the lower limits

Fig. 1. Serum PTH level in all subjects. Serum PTH was measured using a PTH RIA kit (C-assay). The shaded area indicates the normal range of PTH in healthy aged females ($n=75$)²¹⁾ and the dotted line represents the minimal detectable limit of this assay system.⁸⁾ The open and closed circles represent the control and MRC groups, respectively.

of the normal range for the aged. Serum 1,25D levels in 6 of 32 controls exceeded the normal range for the aged.

3) The relationship between serum Ca and calcium regulating hormone levels

The correlation coefficients between serum Ca and serum levels of calcium regulating hormones were calculated. There was a significant correlation between the serum Ca level and 25D ($r=0.505$, $p<0.01$), 24,25D ($r=0.331$, $p<0.05$), 1,25D ($r=0.378$, $p<0.01$) and PTH ($r=-0.331$, $p<0.01$) ($n=49$). There was also a significant correlation between the serum level of 1,25D and both the urinary Ca/creatinine ratio ($r=-0.291$, $p<0.05$) and the serum level of PTH ($r=-0.382$, $p<0.01$) (Table II).

Fig. 2. Serum vitamin D metabolites in all subjects. The shaded area in each column indicates the normal range of each vitamin D metabolite in healthy aged females ($n=75$).²¹⁾ Levels of all three vitamin D metabolites in the MRC group were significantly lower than those in the control group. The open and closed circles represent the control and MRC groups, respectively.

Table II. The Relationships between Serum and Urinary Calcium, and Calcium Regulating Hormones

	25D	24,25D	1,25D	PTH	S-Ca	U-Ca
25D		0.505**	0.395**	-0.311**	0.437**	—
24,25D			—	—	0.331*	—
1,25D				-0.382**	0.378**	-0.291*
PTH					-0.331**	—
S-Ca						—
U-Ca						—

The correlation coefficients between serum and urinary calcium, and calcium regulating hormones in all subjects ($n=49$) are listed. Dotted lines in the table indicate that the correlation coefficients are not significant.

* $p<0.05$, ** $p<0.01$.

DISCUSSION

The factors contributing to calcification of the mitral ring have been investigated for many years. However, the pathogenesis of mitral ring calcification (MRC) has not yet been fully clarified. The most widely accepted opinion is that calcification is usually a result of a degenerative process within the mitral valve.¹⁰⁾ Fulkerson et al¹¹⁾ suggested that a significant association existed between calcification of the mitral anulus and several diseases in which mitral stress may be increased. Roberts and Perloff¹²⁾ reported that aortic stenosis, hypertrophic cardiomyopathy and arterial hypertension might accelerate calcification of the mitral ring. According to these facts, these conditions share one of the following pathophysiologic features: (1) an increased left ventricular intracavity pressure, or (2) an abnormal or increased mitral valve motion.¹⁰⁾

MRC is a condition usually encountered in elderly females, as evidenced by the finding that the prevalence of MRC in autopsy series is 2.5 times higher in females than in males.¹³⁾ Thus, the metabolic abnormalities seen in elderly females might contribute to the pathogenesis of MRC. As is well known, osteoporosis is more frequently seen in elderly females than in elderly males, because calcium metabolism in elderly females is much more unstable than in elderly men.¹⁴⁾ In senile osteoporosis (type II osteoporosis),¹⁴⁾ abnormalities of the calcium regulating hormones have been recognized as a causative factor of decreased bone mass.^{15),16)} On the other hand, MRC is frequently observed in patients with chronic renal failure who had abnormalities in their calcium regulating systems such as secondary hyperparathyroidism or disturbed renal 1,25D production.^{17),18)} From this point of view, we investigated the serum levels of calcium regulating hormones in elderly female patients both with and without MRC.

Our present data clearly demonstrate that there were significantly lower levels of all three vitamin D metabolites in patients with MRC than in controls. In addition, higher serum PTH and lower serum calcium levels were found in the MRC group than in the control group. Significant negative correlations between PTH and calcium, and 1,25D might suggest the presence of secondary hyperparathyroidism in the MRC group, since, if the renal tubular cells in the MRC group retained their normal responsiveness to PTH, high serum levels in 1,25D should be expected.¹⁹⁾ The tendency toward hypocalcemia in the MRC group should be considered as a cause of secondary hyperparathyroidism. The lower level of 1,25D might contribute to the lower serum calcium level, because 1,25D is a known hormonal factor for stimulating intestinal calcium absorption.²⁰⁾ Although the subjects tested

here were adapted to the same dietary conditions, the evidence of a low serum concentration of 25D may indicate that a decreased intestinal absorption rate of vitamin D exists in the MRC group. Recently, it has been reported that paravertebral ligamentous ossification (PVLO) appears to be a frequent complication of hypoparathyroidism.²²⁾ In any type of hypoparathyroidism, it has been well documented that PVLO and other metastatic calcification such as intracranial calcification have frequently been observed.²³⁾ In hypoparathyroidism, patients always show low serum levels of active vitamin D. Thus, it may be argued that hypovitaminosis D could be a more fundamental cause of metastatic calcification than low serum levels of PTH.

However, the exact mechanism(s) of hypovitaminosis D in the MRC group remains unclear. Seasonal variations and the difference in levels of exposure to sunshine were both excluded as possible causes, because our study was carried out throughout the year. As shown in Fig. 2, serum levels of vitamin D metabolites in the patients admitted were sometimes lower than in healthy age-matched controls. Therefore, we have compared the serum levels of vitamin D metabolites in the MRC group to those in a hospitalized control group. On this basis, the difference in the degree of illness could be also excluded as a possible cause of hypovitaminosis D.

In spite of the low serum vitamin D levels in the MRC group, none of the patients showed any apparent clinical evidence of osteomalacia such as high alkaline phosphatase activity, Looser's zone in bone or hyperphosphaturia. The percent tubular reabsorption of phosphate in the MRC group ranged from 80.6 to 96.6% ($89.2 \pm 1.1\%$). However, through study of their X-ray films, 15 of 17 patients from the MRC group were found to have vertebral fracture(s). Therefore, at the least, lower vitamin D levels may contribute to excessive bone loss in these patients.

Previous reports suggested that secondary hyperparathyroidism might play an important role in the pathogenesis of MRC.^{17),18)} However, the present study clearly demonstrates that hypovitaminosis D as well as secondary hyperparathyroidism may play an important role in the formation of metastatic calcification in the mitral ring.

REFERENCES

1. Pomerance A: Pathological and clinical study of calcification of the mitral valve ring. *J Clin Pathol* **23**: 354, 1970
2. Sugiura M, Uchiyama S, Kuwako K, Ohkawa S, Hiraoka K, Ueda K, Shimada H, Otsu S: A clinicopathological study on mitral ring calcification in the aged. *Jpn Heart J* **18**: 154, 1977
3. Korn D, DeSanctis RW, Sell S: Massive calcification of the mitral anulus. A clinicopa-

- thological study of fourteen cases. *New Engl J Med* **267**: 900, 1962
4. DePace NL, Rohrer AH, Kotler MN, Brezin JH, Parry WR: Rapidly progressive massive mitral anular calcification in a patient with chronic renal failure. *Arch Intern Med* **141**: 1663, 1981
 5. Nestico PF, DePace NL, Kotler MN, Rose LI, Brezin JH, Swartz C, Mintz GS, Schwartz AB: Calcium and phosphorus metabolism in dialysis patients with and without mitral anular calcium. *Am J Cardiol* **51**: 497, 1983
 6. Okimoto T, Iwasaki T, Kamata C, Ohkawa S, Hashimoto H, Matsushita T, Ueda K, Sugiura M: Echo- and phonocardiographic studies of mitral ring calcification. *Cardiovasc Sound Bull* **5**: 707, 1975
 7. Hirschfeld DS, Emilson BB: Echocardiogram in calcified mitral anulus. *Am J Cardiol* **36**: 354, 1975
 8. Shiraki M, Kawada N, Akiguchi I, Yamauchi H, Kojima A, Orimo H: 1-34 human parathyroid hormone radioimmunoassay: properties of antiserum to synthetic 1-34 human parathyroid hormone and its clinical application. *Endocrinol Japon* **28**: 239, 1981
 9. Ishizuka S, Naruchi T, Hashimoto Y, Orimo H: Radioreceptor assay for 1 alpha-24(R), 25 trihydroxyvitamin D₃ in human serum. *J Nutr Sci Vitaminol* **27**: 71, 1981
 10. Sell S, Scully RE: Aging changes in the aortic and mitral valves. *Am J Pathol* **46**: 345, 1965
 11. Fulkerson PH, Beaver BM, Auseon JC, Graber HL: Calcification of the mitral anulus: etiology, clinical associations, complications and therapy. *Am J Med* **66**: 967, 1979
 12. Roberts WC, Perloff JK: Mitral valve disease. *Ann Int Med* **77**: 939, 1972
 13. Suzuki J, Ohkawa S, Sugiura M, Sakai M, Chida H, Watanabe C, Matsushita S, Ueda K, Kuramoto K, Takahashi T: Mitral valvular disease secondary to mitral ring calcification: a clinicopathological study. *J Cardiol* **15**: 1109, 1985 (in Japanese with English abstract)
 14. Riggs BL, Melton LJ III: Involutional osteoporosis. *New Engl J Med* **314**: 1676, 1986
 15. Orimo H, Shiraki M: Role of calcium regulating hormones in the pathogenesis of senile osteoporosis. *Endocr Japon* **SR1**: 1, 1979
 16. Teitelbaum SL, Rosenberg EM, Richardson CA, Avioli LV: Histological studies of bone from normocalcemic postmenopausal osteoporotic patients with increased circulating parathyroid hormone. *J Clin Endocrinol Metab* **42**: 537, 1976
 17. Berson SA, Yarrow RS: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia, and bronchogenic carcinoma. *Science* **154**: 907, 1966
 18. Mawer EB, Backhouse J, Taylor CM, Lumb GA, Stanbury SW: Failure of formation of 1,25-dihydroxycholecalciferol in chronic renal insufficiency. *Lancet* **I**: 626, 1973
 19. Eisman JA, Wark JD, Prince RL, Moseley JM: Modulation of plasma 1,25-dihydroxyvitamin D in man by stimulation and suppression tests. *Lancet* **II**: 931, 1979
 20. Spencer R, Charman M, Wilson P, Lawson E: Vitamin D-stimulated intestinal calcium absorption may not involve calcium-binding protein directly. *Nature* **263**: 161, 1976
 21. Shiraki M, Akiguchi I, Ito H, Ishizuka S, Orimo H: Effect of aging on bone mineral content. Part IV. Normal range and physiological importance of serum vitamin D metabolites and parathyroid hormone levels in healthy aged females. *Jpn J Geriatr* **22**: 420, 1985 (in Japanese with English abstract)
 22. Okazaki T, Takuwa Y, Yamamoto M, Matsumoto T, Igarashi T, Kurokawa T, Ogata E: Ossification of the paravertebral ligaments: a frequent complication of hypoparathyroidism. *Metabolism* **33**: 710, 1984
 23. Aurbach GD, Marx SJ, Spiegel AM: Parathyroid hormone, calcitonin and the calciferols. *in* Textbook of Endocrinology, ed by Williams RH, 6th Edition, WB Saunders, Philadelphia, London, Toronto, p 922, 1981