CHAPTER IV

RESULTS OF THE STUDY

From January 2004 to October 2004, 72 postmenopausal women age 65 years or more, who came to Osteoporosis Clinic at Department of Orthopaedics, Phramongkutklao Army Hospital were enrolled to this study. All of them were able to walk alone and none of them engaged in regular physical exercise programs.

Table 4.1 Baseline and demographic data in all participants (72 cases)

		Mean	SD	Min-Max
Age (yr)		70.6	4.3	65-84
Weight (kg)		60.8	6.7	45.8-71.0
Serum 25(OH)D_3 (ng/ml)		27.99	7.67	12.00-70.83
Serum iPTH (pg/ml)		45.14	19.23	15.04-94.50
VDR genotype [N (%)]				
BB	4 (5.6%)			
Bb	18 (25.0%)			
bb	50 (69.4%)			
Muscle strength at 30°/sec (N-m)		18.95	7.36	6.40-44.00
Muscle strength at 60°/sec (N-m)		17.44	8.55	4.70-55.15

Table 4.1 shows the baseline and demographic data of all participants in this study. Participants' age varied from 65 to 84 years old with a mean age of 70.6 years. The average body weight was 60.8 kilograms. Mean serum $25(OH)D_3$ was 27.99 ng/ml and mean serum intact PTH was 45.14 pg/ml. Mean quadriceps muscle strength measure by isokinetic dynamometer at 30°/sec was 18.95 ± 7.36 N-m, and at 60°/sec was 17.44 ± 8.55 N-m. The frequency distribution of VDR genotype in this study was BB 5.6 %, Bb 25 % and bb 69.4 %. If we categorized these baseline characteristics according to the serum level of $25(OH)D_3$ into a group of serum $25(OH)D_3 > 30$ ng/ml and a group of serum $25(OH)D_3 \leq 30$ ng/ml, the distribution of these baseline data were shown as table 4.2. According to the study by Soontrapa et al[35], vitamin D insufficiency or hypovitaminosis D was defined as the population who have serum $25(OH)D_3$ equal or lower than 30 ng/ml. In this study the percentage of hypovitaminosis D was very high as 63.9 % (46 in 72 cases). The mean age, weight, serum iPTH and initial muscle strength at both angular velocity were not difference between two groups. There were slightly more frequency of bb genotype in the group of serum $25(OH)D_3 \leq 30$ ng/ml.

Table 4.2 Baseline and demographic data expressed as mean ± SD in all participants (72 cases) categorized by level of serum $25(OH)D_3$ into group of > 30 ng/ml and group of ≤ 30 ng/ml

	$25(OH)D_3$ > 30 ng/ml	$25(OH)D_3$ ≤ 30 ng/ml
Number	26	46
Age (yr)	69.8 ± 4.2	71.0 ± 4.3
Weight (kg)	60.93 ± 5.65	60.78 ± 7.36
Serum iPTH (pg/ml)	47.64 ± 19.62	43.73 ± 19.08
VDR genotype [N (%)]		
BB	2 (7.7%)	2 (4.3%)
Bb	8 (30.8%)	10 (21.7%)
bb	16 (61.5%)	34 (73.9%)
Muscle strength at 30°/sec (N-m)	18.73 ± 7.25	19.07 ± 7.5
Muscle strength at 60°/sec (N-m)	17.63 ± 9.54	17.33 ± 8.04

There were 46 elderly subjects who have serum $25(OH)D_3$ ≤ 30 ng/ml, and forty-two of them willing to participate into this study. These women were randomly allocated into either treatment (alfacalcidol 0.5 μg/d + calcium carbonate 1,500 mg/d) and control (placebo + calcium carbonate 1,500 mg/d) groups by simple randomization. There were two subjects withdrawn from the study before it had been finished. One subject discontinued the intervention drug at an early state (< 4 weeks), another subject sustained an ankle fracture, so the number of subjects who received the second muscle strength measurement at 12 weeks were 40. After open the concealment, we found that the two dropped out subjects were in the placebo group, so the number of subjects in placebo group were 19, while the number of subjects in treatment group were 21.

Table 4.3 Baseline, demographic data expressed as mean ± SD, and result of improvement in muscle strength* in participants group ≤ 30 ng/ml who enrolled in experimental RCT.

		Placebo	Alfacalcidol
Number	at first enrollment	21	21
	at final	19(2 dropped out)	21
Age (yr)		70.6 ± 3.8	70.9 ± 4.0
Weight (kg)		61.28 ± 7.94	60.70 ± 6.66
Serum 25(OH)D_3 (ng/ml)		23.82 ± 3.24	24.71 ± 4.57
Serum iPTH (pg/ml)		43.86 ± 19.27	41.72 ±17.26
VDR genotype [N (%)]			
	BB	--	2 (9.5%)
	Bb	4 (19%)	5 (23.8%)
	bb	17 (81%)	14 (66.7%)
Baseline muscle strength at 30°/sec (N-m)		19.20 ± 8.80	19.63 ± 6.10
Baseline muscle strength at 60°/sec (N-m)		18.01 ± 9.06	17.43 ± 7.19
Muscle strength at 12 wk, 30°/sec (N-m)		16.09 ± 5.28	20.28 ± 8.84
Muscle strength at 12 wk, 60°/sec (N-m)		15.05 ± 6.13	20.32 ± 8.56
Mean percent difference from baseline at 30°/sec (% ± SD)		-8.00 ± 29.9	3.17 ± 29.9
Mean percent difference from baseline at 60°/sec (% ± SD)		-5.73 ± 46.5	27.13 ± 57.1

Randomized controlled study result

Table 4.3 shows the baseline and the post-intervention results of this study. Mean age, weight, serum 25(OH)D_3, serum iPTH and initial muscle strength were not significant difference. The VDR genotype in placebo group had more frequency in bb population than in treatment group. After 12 weeks of intervention, there was an

improvement of muscle strength in treatment in both angular velocity compared to the placebo group, which the muscle strength decreased in both angular velocity. We use Analysis of Co-variance (ANCOVA) to compares the results of muscle strength at 12 weeks between Alfacalcidol and placebo group. In this model, we set the baseline muscle strength as a covariate. Result from the ANCOVA show statistical significant improvement of muscle strength($p= 0.025$ in 30°/sec and $p= 0.002$ in 60°/sec) in Alfacalcidol group compare to placebo in both angular velocity (Table 4.4 and 4.5). Figure 4.1 show the box-plot distribution of muscle strength at 12 weeks and muscle strength at baseline show improvement in muscle strength in both angular velocity.

Table 4.4 ANCOVA table Test of between-subject effects: dependent variable is muscle strength at 12 weeks, 30°/sec

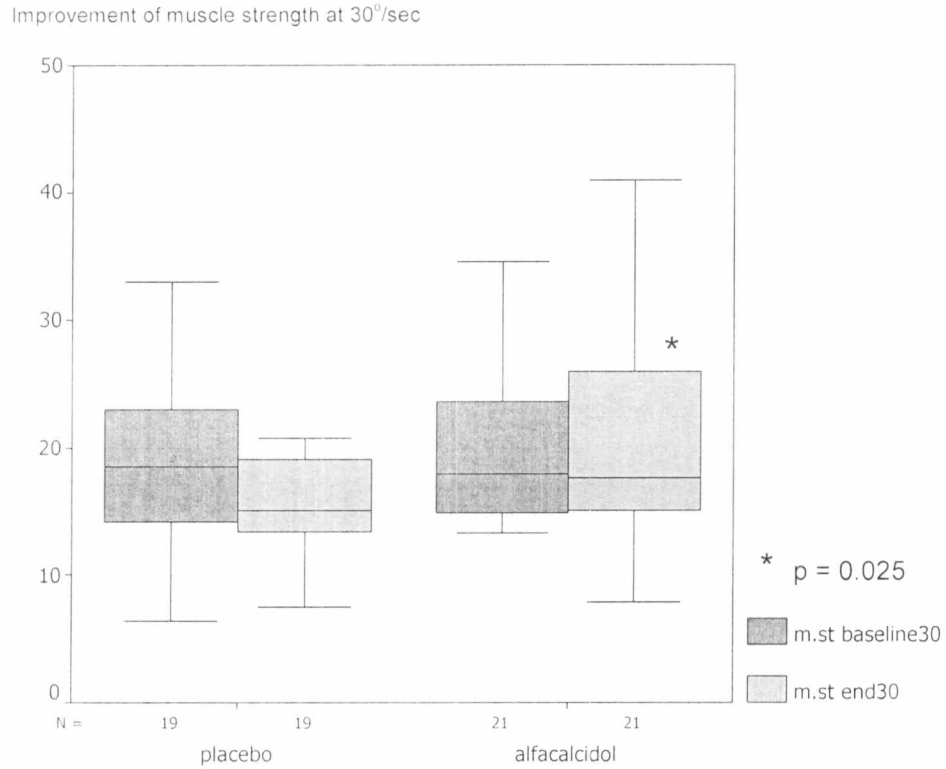
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1149.977*	2	574.988	19.498	.000
Intercept	152.484	1	152.484	5.171	.029
MSB30	974.731	1	974.731	33.053	.000
DRG	160.261	1	160.261	5.434	.025
Error	1091.139	37	29.490		
Total	15622.080	40			
Corrected Total	2241.116	39			

* R Squared = .513 (Adjusted R Squared = .487), MSB30 = baseline muscle strength at 30°/sec
DRG = Alfacalcidol group significant improvement (p=0.025)

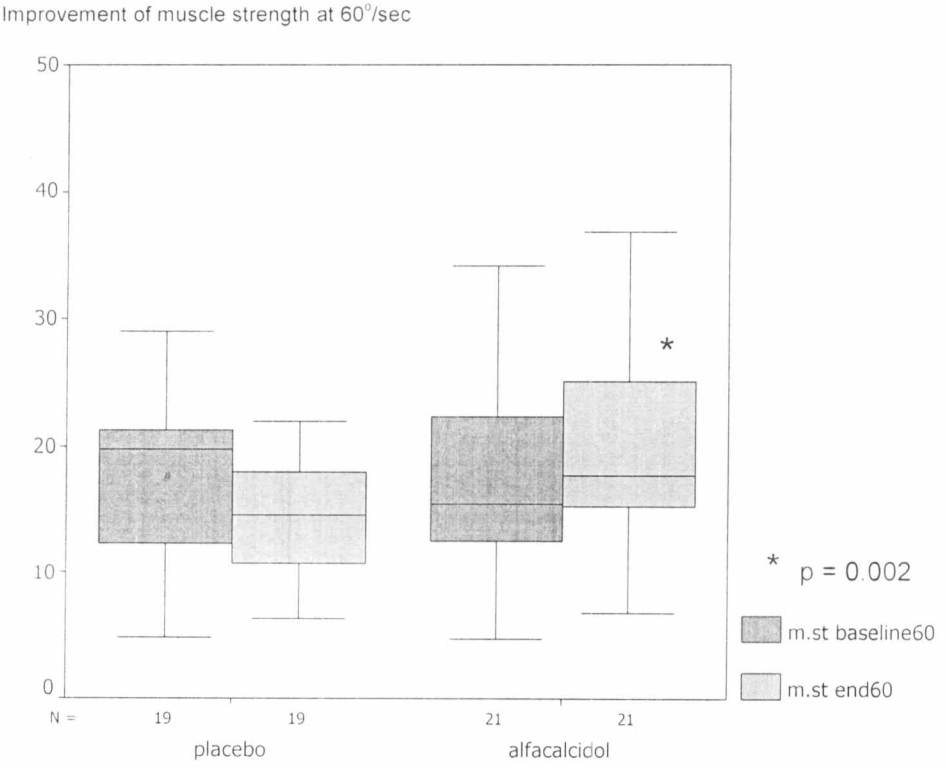
Table 4.5 ANCOVA table Test of between-subject effects: dependent variable is muscle strength at 12 weeks, 60°/sec

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1271.603*	2	635.801	20.483	.000
Intercept	311.448	1	311.448	10.034	.003
MSB60	994.167	1	994.167	32.028	.000
DRG	338.184	1	338.184	10.895	.002
Error	1148.486	37	31.040		
Total	15120.403	40			
Corrected Total	2420.088	39			

* R Squared = .525 (Adjusted R Squared = .500), MSB60 = baseline muscle strength at 60°/sec
DRG = Alfacalcidol group significant improvement (p=0.002)



m.st baseline30 = muscle strength at baseline at 30°/sec, m.st. end30 = muscle strength at12 weeks at 30°/sec



m.st baseline60 = muscle strength at baseline at 60°/sec, m.st. end60 = muscle strength at12 weeks at 60°/sec

Figure 4.1 Improvement in muscle strength in treatment versus placebo group in both angular velocity (30°/sec and 60°/sec)

Correlation study of baseline data to muscle strength

We calculated Pearson's correlation coefficient (r) for the correlation study between serum 25(OH)D_3 and muscle strength in both 30°/sec and 60°/sec angular velocity. We could not detect any statistical significant correlation between serum 25(OH)D_3 and muscle strength in both angular velocities. They also not statistically significant, when take out one case of the outliner from the data in both angular velocity. And again, when we calculated by take out all the data that have serum 25(OH)D above 30 ng/ml, it still no statistically significant. Distribution of data was shown in Figure 4.2. Correlation between serum iPTH to the baseline muscle strength in both angular velocities also not statistically significant (Figure 4.3). There was a negative correlation between serum 25(OH)D_3 and serum intact PTH(Figure 4.4), but it was not statistically significant also ($r = -0.114$; $p = 0.35$). All of these data were summary and shown in table 4.4. About the VDR genotype, the numbers of BB in our subject are too small, so we do not calculate the correlation for these. We calculated the correlation between serum 25(OH)D_3 and muscle strength in the group who have VDR genotype bb and in the group who have VDR genotype bb and Bb, no correlation were found.(table 4.4)

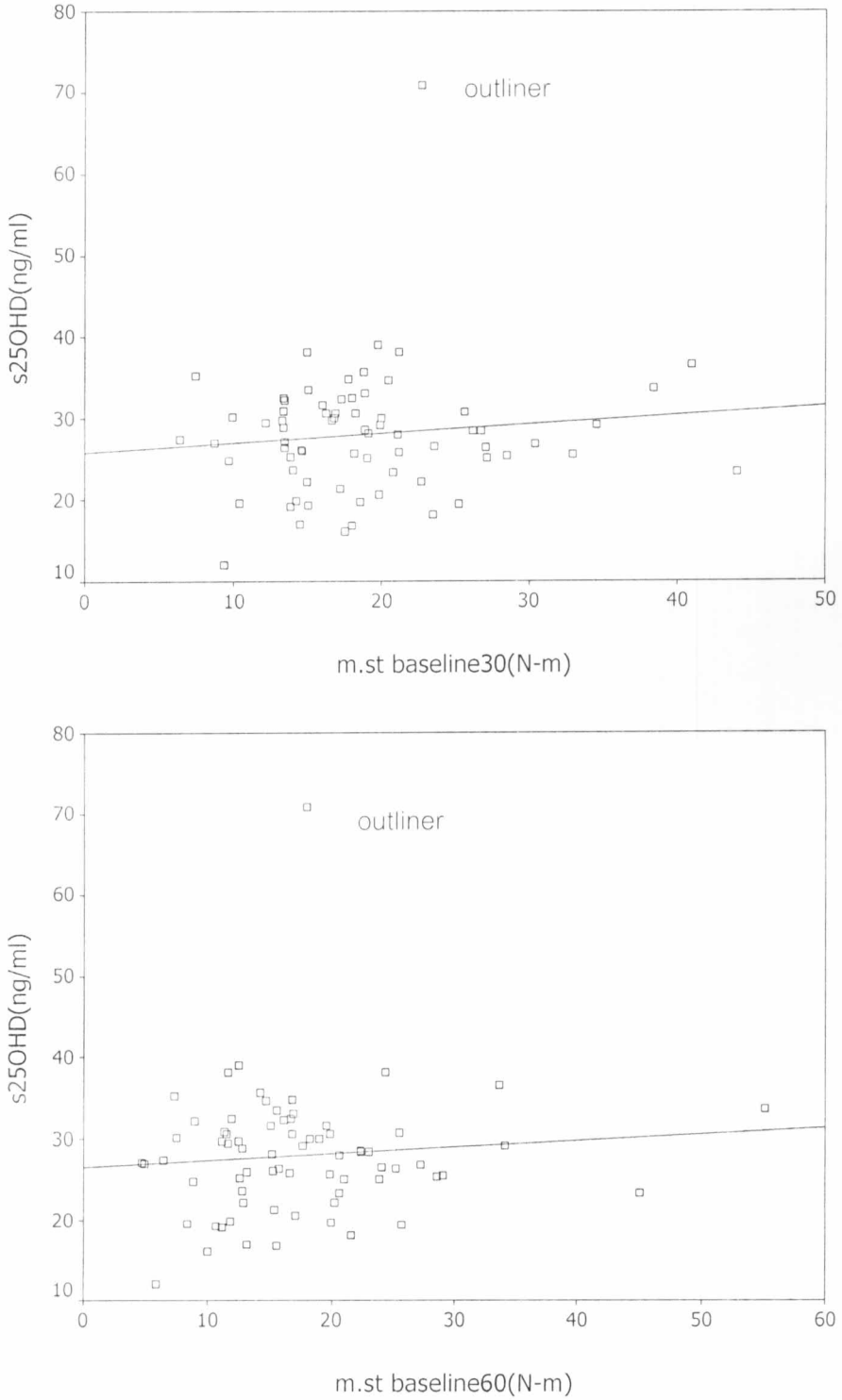


Figure 4.2 Correlation between serum 25(OH)D_3 and muscle strength in both angular velocity (30°/sec and 60°/sec)

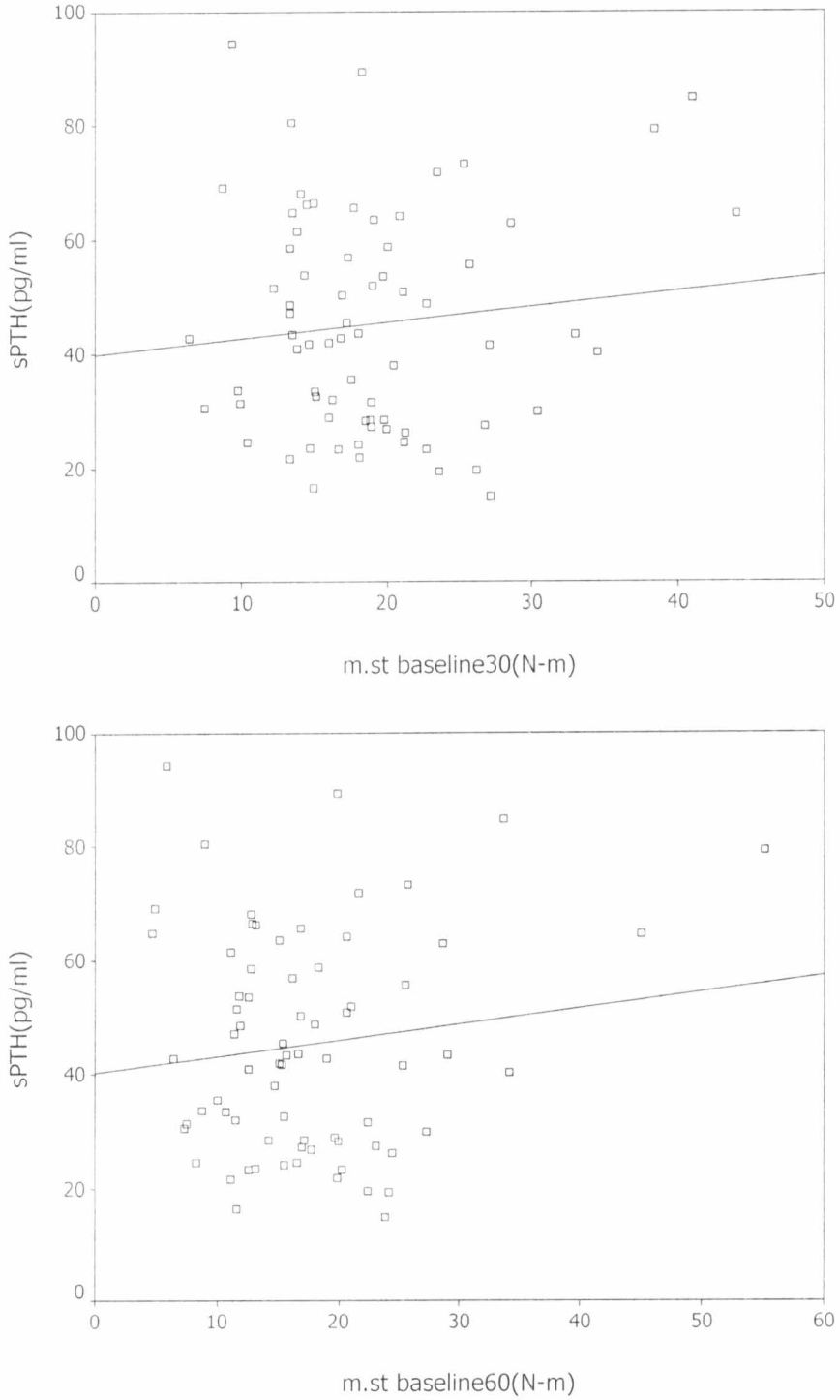


Figure 4.3 Correlation between serum iPTH and muscle strength in both angular velocity (30°/sec and 60°/sec)

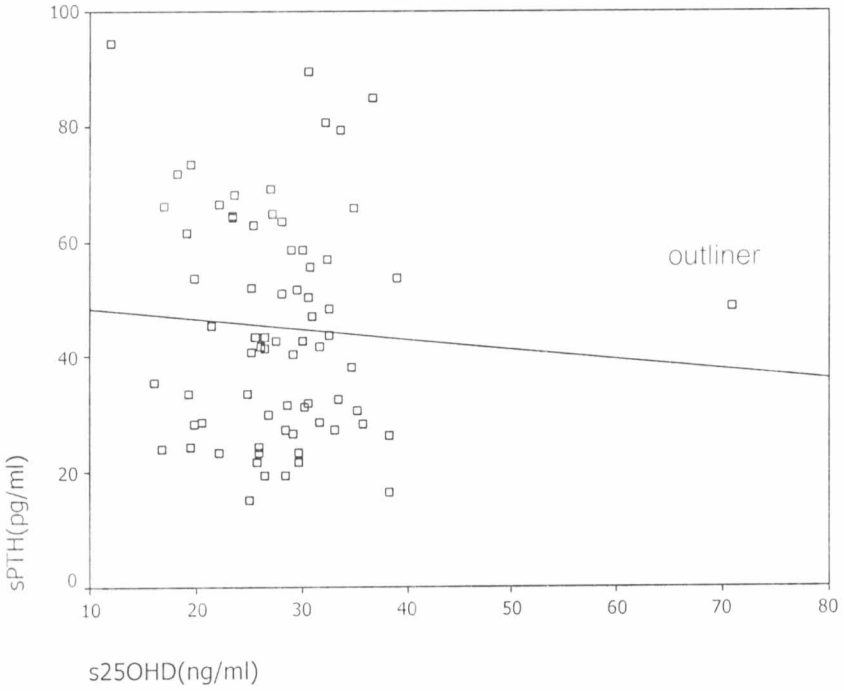


Figure 4.4 Correlation between serum iPTH and serum 25(OH)D_3

Table 4.6 Correlation study between baseline muscle strength of both two angular velocity with serum 25(OH)D_3 and serum iPTH

	condition (N)	Baseline muscle strength at 30°/sec	Baseline muscle strength at 60°/sec
Serum 25(OH)D_3	all cases (N=72)	r = 0.107 (p=0.37)	r = 0.089 (p=0.45)
Serum 25(OH) D_3	-1 outliner (N=71)	r = 0.09 (p=0.45)	r = 0.114 (p=0.35)
Serum 25(OH) D_3	$\leq$ 30 ng/ml (N=46)	r = 0.154 (p=0.31)	r = 0.190 (p=0.21)
Serum 25(OH) D_3	bb genotype (N=50)	r = 0.146 (p=0.31)	r = 0.131 (p=0.35)
Serum 25(OH) D_3	bb and Bb genotype (N=68)	r = 0.105 (p=0.40)	r = 0.087 (p=0.48)
Serum iPTH	all cases (N=71)	r = 0.106 (p=0.37)	r = 0.127 (p=0.29)
Serum iPTH	-1 outliner (N=71)	r = 0.105 (p=0.38)	r = 0.127 (p=0.29)

Result regarding adverse events and complications were summarized in table 4.5. No serious adverse events reported. All subjects except two of them, were continued in this study till its finish. The most common side effect reported in literature about the treatment of active vitamin D metabolites is hypercalcemia, but rarely report in trials use of alfacalcidol. The two dropped out subjects; one had moderately dizziness after administration of intervention drugs, and she intended to quit the drugs. The other was sustained an ankle fracture and was discontinue to follow up this study. Both subjects were randomized to the placebo group, so there were no any significant adverse events in treatment group.

Table 4.7 Adverse events that potentially related to the investigation drugs reported by subjects: number of cases(percent)

	Placebo	Alfacalcidol
Pruritus	1 (5.3 %)	0
Dyspepsia	2 (10.5 %)	1 (4.8 %)
Mild headache	1 (5.3 %)	1 (4.8 %)
Mild dizziness	1 (5.3 %)	0
Mild myalgia	0	1 (4.8 %)