Efficacy and Safety Analysis of Alfacalcidol Combined with Cinacalcet in the Treatment of Secondary Hyperparathyroidism in Patients with Chronic Renal Failure in Maintenance Hemodialysis

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To investigate the efficacy of alfacalcidol combined with cinacalcet as treatment on secondary hyperparathyroidism in patients with chronic renal failure who are currently in maintenance hemodialysis. Patients in maintenance hemodialysis with chronic renal failure in Zhuji People's Hospital were selected as the research subjects and randomly divided into the control group and the observation group, 50 cases in each. The patients in the control group were treated with alfacalcidol capsules and the patients in the observation group were treated with alfacalcidol capsules combined with cinacalcet tablets. Both groups were treated for 3 consecutive mo. The clinical efficacy, serum factors (calcium, phosphorus, intact parathyroid hormone, alkaline phosphatase, beta-type I collagen C-terminal peptide and procollagen type I N-terminal propeptide) levels, quality of life, parathyroid volume and Ki-67 protein positive rate were compared between the two groups after treatment. After 3 mo of treatment, the total effective rate (94 %) of the observation group was significantly higher than that of the control group (74 %) and the difference between the two groups was statistically significant ($p<0.05$). Compared with the control group, the levels of serum factor indexes in the experimental group were improved after treatment and the difference was statistically significant ($p<0.05$). The parathyroid volume of the patients in the two groups decreased and the experimental group was significantly lower than the control group after treatment ($p<0.05$). Further research found that the positive rate of Ki-67 protein in the experimental group was significantly lower than that before treatment and the control group after treatment ($p<0.05$). This combination of medication could improve the efficacy on different aspects like reduce the volume of parathyroid glands, optimize the quality of life of patients and improve the safety of treatment.

Key words: Alfacalcidol, cinacalcet, chronic renal failure, hyperparathyroidism

The incidence of Chronic Kidney Disease (CKD) is on the rise worldwide and it has become an important disease that threatens well-being of mankind. A meta-analysis of prevalence studies in 44 countries estimated the global prevalence of CKD at 13.4 %[1]. Secondary Hyperparathyroidism (SHPT) is a common and severe complication in patients with CKD, characterized by decreased 1,25-dihydroxyvitamin D and consequent activation of the vitamin D receptor, serum abnormal Calcium (Ca) and Phosphorus (P) levels, parathyroid hyperplasia, intact Parathyroid Hormone (iPTH). Poorly controlled SHPT contribute to the development and progression of mineral and bone abnormalities in CKD patients[2,3]. In dialysis patients, the severity of SHPT tends to increase, often accompanied by symptoms such as bone and joint pain, pruritus, fractures and mental disorders[4], which seriously affects their quality of life. Clinical reports show that SHPT is closely related to the high incidence of cardiovascular accidents and death, and has a serious impact on the survival of patients[5,6]. For a long time, alfacalcidol and its analogs have been the core drugs in the treatment of CKD SHPT. However, these drugs can increase the intestinal absorption of Ca and P, resulting in increased

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serum Ca and P concentrations[7]. Furthermore, the effect of vitamin D analogs is weakened when parathyroid hyperplasia progresses to parathyroid nodules. Therefore, when SHPT gradually progresses from moderate to severe SHPT, the efficacy of traditional drug therapy is reduced. Cinacalcet is a Ca-sensing receptor agonist, which mainly acts on the parathyroid Ca receptor. It could treat SHPT *via* reduce the serum Ca concentration. Numerous clinical studies have confirmed that cinacalcet can effectively reduce iPTH levels in patients with SHPT, even those who are resistant to vitamin D therapy[8,9]. Studies have also shown that cinacalcet can reduce the volume of hyperplasic parathyroid glands lead to reduced number of parathyroidectomy[10]. Therefore, this study evaluates the safety of alfacalcidol combined with cinacalcet as treatment for SHPT in patients with chronic renal failure and is in maintenance hemodialysis to and compared the efficacy with the conventional alfacalcidol treatment. 100 patients with chronic renal failure and currently in maintenance hemodialysis in Zhuji People's Hospital from January 2020 to January 2021 were selected. Inclusion criteria[11] includes regular hemodialysis for >6 mo; patients with SHPT (iPTH>300 pg/ml, normal value 16-62 pg/ml) in maintenance dialysis. Exclusion or withdrawl criteria includes drug allergy; severe cardiovascular disease; risk of seizures; severe liver dysfunction; severe gastrointestinal bleeding; gastrointestinal ulcers; malignancy; severe hypocalcemia (<1.9 mmol/l), hypercalcemia (>2.7 mmol/l), hyperphosphatemia (>2.9 mmol/l) and patients who cannot tolerate cinacalcet. All patients were aware of the content and purpose of this study and voluntarily signed the informed consent, which was approved by the Ethics Committee of Zhuji People's Hospital. 100 patients were randomly divided into control group and experimental group, 50 cases in each group. Control group patients took alfacalcidol capsules 0.25 μg/d. The drug dose was adjusted according to the results of regular testing every 3 w. The maximum dose of the pill is 0.5 μg/d and the treatment is continued for 3 consecutive mo. Experimental group patients were given oral cinacalcet tablets 25 mg/d on the basis of the control group and the dose was adjusted according to the patient's tolerance and actual condition. The maximum dose of cinacalcet was 75 mg/d and the drug was taken for 3 consecutive mo. All patients in both group received a low-phosphorus and low-protein diet. In clinical efficacy after treatment, if the patient's symptoms such as sleep, bone pain, arthralgia and itching disappeared or were significantly relieved and the iPTH level decreased by >75 % compared with that before treatment is consider remarkably effective. After treatment, if the iPTH level is reduced by 25 % to 75 % compared with that before will be consider effective[12]. If the symptoms are slightly relieved or not relieved and the iPTH level is decreased <25 % compared with before will be considered non-effective. Total effective rate=(number of markedly effective cases+number of effective cases)/total number of cases×100 %. Comparison of serum concentration level of iPTH, Ca, P, Alkaline Phosphatase (ALP), beta-type I Collagen C-Terminal Peptide (β-CTX), changes in the peptide and Procollagen Type I N-Terminal Propeptide (PINP) before and after 3 mo of treatment in the two groups. Quality of life evaluation was assessed by the Brief Health Status Survey Scale (SF-36)[13], which included health status, physical pain, mental health, energy, etc. Each dimension was recorded as 0-100 points, the higher the total score indicates better quality of life. Parathyroid volume includes the length, width and thickness of the parathyroid glands in the two groups were measured by cervical ultrasound using a color Doppler ultrasonograph before and after treatment and the volume of the parathyroid glands in the two groups was compared. The parathyroid tissue of all subjects was extracted and the expression of Ki-67 was detected by immunohistochemistry. Staining results were scored independently according to the same scoring criteria by two experienced clinicopathologists without knowledge of the section data. The results were judged by a double scoring method; 10 clearer fields of view were selected for each section to count the number of positively stained cells and the staining intensity was scored based on the staining characteristics of most cells (the staining depth should be compared with the background staining); none staining is 0 points, light yellow is 1 point, brown is 2 points and tan is 3 points; according to the ratio of positive cells (i.e., the average number of positive cells in 5 high-magnification fields (400×) randomly selected in each case); the number of positive cells <25 % was scored as 0 point; 15 %-50 % was scored as 1 point; 50 %-75 % was scored as 2 points; the number of positive cells ≥75 % was scored as 3 points. Take the product of the two sets of scores, and score ≤3 points as negative and >3 points as positive. Statistical Package for the Social Sciences (SPSS) 23.0 statistical software was used to analyze the data.

Measurement data were expressed as mean±standard deviation ($\bar{x}$±s) and were analyzed by t test; enumeration data were expressed as rate percent (%) and differences were compared by Chi-square (χ^2) test. $p<0.05$ is considered statistically significant. In this study, the subjects were randomly divided into an experimental group and a control group, with 50 cases in each. Among the subjects, there were 24 males and 26 females in the experimental group, ranging in age from 35 y to 70 y old, with an average age of (43.50±6.19) y, dialysis time of 6 mo to 80 mo and an average dialysis time of (35.94±13.11) mo; there were 22 males and 28 females in control group, aged 32 y to 72 y, with an average age of (45.02±6.90) y, dialysis time from 7 mo to 84 mo and an average dialysis time of (36.82±14.37) mo. There were no significant differences in gender, age and dialysis time between the two groups ($p>0.05$), which were comparable. After treatment, the efficacy of treatment in the control group mainly were effective (14.00 %), while the efficacy in experimental group were remarkably effective (82.00 %), followed by the effective (12.00 %). Further analysis found that the total efficiency of the experimental group was 94.00 %. (47/50), significantly higher than 74 % (74/50) in the control group and the difference between the two groups was statistically significant ($\chi^2=7.44$, $p=0.007$). The results are shown in Table 1. Before treatment, there was no significant difference in serum factor indexes between the two groups ($p>0.05$). Both group has a lower levels of serum Ca, P, iPTH, ALP, β-CTX and PINP after than before treatment ($p<0.05$). The after treatment serum levels of P, iPTH, ALP, β-CTX and PINP in the experimental group were lower than in the control group and the differences were statistically significant ($p<0.05$). The results of the study showed that both treatments effectively reduced the levels of serum factor indexes and the efficacy in experimental group is higher than in control groups for serum factor aspect. The results are shown in Table 2. Before treatment, the difference between the scores of all dimensions of quality of life in the two groups was relatively low with no statistically significance ($p>0.05$). The life of the patients in both groups improved after treatment including the scores of health status, physical pain, mental health and energy. Moreover, the health status of the experimental group (44.09±11.50), physical pain (45.66±10.50±10.07±10.16), mental health (49.08±8.93) and energy (49.20±8.86) scores were higher than those of the control group in terms of health status (32.76±8.49), physical pain (31.56±8.53), mental health (36.30±8.31) and energy (39.66) ±8.75), and the difference was statistically significant ($p<0.05$). The results are shown in Table 3. Before treatment, the volume of parathyroid glands in the observation group and the control group were (1.78±0.31) cm^3 and (1.69±0.33) cm^3 respectively, with no statistically significant difference ($t=1.42$, $p=0.158$). After treatment, the volume of parathyroid glands in the observation group and the control group decreased, which were (0.94±0.22) cm^3 and (1.34±0.20) cm^3, respectively, and the difference was statistically significant ($t=9.50$, $p<0.001$) and ($t=9.50$, $p<0.001$). To further explore the proliferation of parathyroid cells, Ki-67 immunohistochemical staining was performed. In the parathyroid gland section, the nuclei were stained uniform brown and Ki-67 protein was stained in the nucleus. The positive rates of Ki-67 protein in the experimental group before and after treatment were 62 % (31/50) and 36 % (18/50), respectively. The positive rates of Ki-67 protein in the control group before and after treatment were 68 % (34/50) and 58 % (29/50), respectively. There was no significant difference in the positive rate of Ki-67 protein between the two groups before treatment (62 % *vs.* 68 %, $\chi^2=0.40$, $p=0.529$) and there was a significant difference in the positive rate of Ki-67 protein between the two groups after treatment (36 % *vs*. 58 %, $\chi^2=4.88$, $p=0.028$). Chronic Kidney Disease-Mineral Bone Abnormality (CKD-MBD) is the most common complication in maintenance hemodialysis patients. It characterized as abnormal metabolic indicators like high blood Ca, blood phosphorus, blood iPTH and abnormal bone metabolism and calcification defense. SHPT is one of the most common and fatal complication of CKD-MBD, which increases fractures, cardiovascular mortality and all-cause mortality. The traditional treatment of SHPT is phosphorus binders and active vitamin D such as alfacalcidol or calcitriol. It could reduce iPTH synthesis by inhibiting PTH gene transcription and sensitize Ca^{2+} receptors and vitamin D receptors. Restoring the Ca^{2+} setting point can balance bone metabolism, thereby reducing the occurrence of SHPT. Alfacalcidol is effective in the initial treatment resulting in reducing the iPTH level, but with the progression of the disease, hypocalcaemia often occurs in the later stage, which significantly increases the Ca load of the patient, which makes it difficult for the patient to reach the blood iPTH standard, lead to progression of SHPT. Several studies[14,15] have shown that cinacalcet, as a calcimimetic, can allosterically modulate Ca-sensing receptors by the drug, increasing the sensitivity of parathyroid cells to extracellular Ca^{2+}. Thus, it could

reduce the production and secretion of iPTH, reduce the concentration of blood Ca and blood phosphorus, so that the volume of hyperplastic parathyroid adenomawill decreases to a certain extent. Hypocalcaemia, however, is not uncommon with cinacalcet therapy and its effect on serum Ca levels can be counteracted by co-administration of alfacalcidol or calcitriol[16]. Alfacalcidol is a non-selective vitamin D receptor agonist, which can increase intestinal Ca and P absorption and increase bone Ca and P mobilization, resulting in increased serum Ca and P levels, which may increase the risk of ectopic vascular calcification and cardiovascular mortality[17]. The combined application of the two can synergistically reduce iPTH and reduce the incidence of hypocalcemia, hypercalcemia and hyperphosphatemia. In this study, the control group and the experimental group were established to explore the efficacy of combined of alfacalcidol and cinacalcet compared with single alfacalcidol treatment. The results of this study showed that after treatment, the total effective rate (94.00 %) of the experimental group was significantly higher than that of the control group (74.00 %) and the difference between the groups was statistically significant ($p<0.05$). After treatment, the levels of serum factor indexes Ca, P, iPTH, ALP, β-CTX and PINP in the experimental group were significantly lower than those before treatment and were better than those in the control group, and the difference between the groups was statistically significant ($p<0.05$). The evaluation dimensions of quality of life, including health status, physical pain, mental health and energy, were significantly better than those before treatment and the control group. After treatment, the volume of parathyroid glands in both groups was reduced with more significant effect in experimental group ($p<0.05$). Further research found that the positive rate of Ki-67 protein in the experimental group after treatment was significantly lower than that before treatment and the reduction is more significant than control group ($p<0.05$). The result suggested that the combined treatment of alfacalcidol and cinacalcet can reduce the expression of Ki-67 protein, thereby greatly reducing the volume of parathyroid glands. According to the early study[18], cells in the proliferative stage will produce Ki-67. The higher the positive rate of Ki-67, the more active the cell proliferation, i.e., the greater the possibility of malignant lesions. Therefore, the combined effect of the above two drugs on parathyroid cells proliferation has a certain inhibitory effect. The results of this study suggest that alfacalcidol combined with cinacalcet can more effectively control the levels of Ca, P, iPTH, ALP, β-CTX and PINP in patients with SHPT and reduce the volume of parathyroid glands, improve their quality of life and improve the total efficacy of treatment. This paper preliminarily showed that alfacalcidol combined with cinacalcet is safe and effective which is worthy of further clinical research. The limitation of this study is that the SF-36 scale only reflects the improvement of the patient's health status, physical pain, mental health and energy total score, and does not comprehensively analyze and evaluate the patient's quality of life. Since the sample size of this study is small, further studies with larger sample size and longer follow-up time, such as multicenter clinical trials are needed to support this finding.

TABLE 1: COMPARISON OF CLINICAL EFFICACY BETWEEN TWO GROUPS OF PATIENTS AFTER TREATMENT [n (%)]

Efficacy	Experimental group (n=50)	Control group (n=50)	χ^2	p value
Remarkably effective	41 (82.00)	30 (60.00)	-	-
Effective	6 (12.00)	7 (14.00)	-	-
Non-effective	3 (6.00)	13 (26.00)	-	-
Total effective rate	47 (94.00)	37 (74.00)	7.44	0.007

TABLE 2: COMPARISON OF SERUM FACTOR INDEXES BETWEEN TWO GROUPS OF PATIENTS (x±s)

Subsets	Before treatment	After treatment	t value	p value	Before treatment	After treatment	t value	p value
	Ca (mmol/l)				P (mmol/l)			
Experimental group	2.16±0.50	1.77±0.54	3.75	<0.001	2.37±0.44	1.73±0.46	7.11	<0.001
Control group	2.21±0.35	1.88±0.56	3.53	<0.001	2.44±0.55	2.43±0.57	0.089	0.929
t value	0.62	-1			-0.72	6.73		
p value	0.538	0.32			0.473	<0.001		
Subsets	**iPTH (pg/ml)**				**ALP (U/l)**			
Experimental group	1043.00±263.20	319.20±102.9	18.11	<0.001	153.60±47.95	96.06±38.24	6.63	<0.001
Control group	915.30±276.20	427.40±136.70	11.195	<0.001	132.70±62.05	120.50±45.99	1.117	0.267
t value	-1.37	4.47			-1.88	2.89		
p value	0.12	<0.001			0.063	0.005		
Subsets	**ß-CTX (ng/ml)**				**PINP (ng/ml)**			
Experimental group	3.26±1.03	2.13±0.83	6.04	<0.001	180.20±62.66	111.10±50.48	2.51	0.014
Control group	3.04±0.89	2.64±0.69	6.07	<0.001	195.00±66.65	151.60±58.39	3.463	<0.001
t value	-1.14	3.36			1.15	3.7		
p value	0.257	0.001			0.255	<0.001		

TABLE 3: COMPARISON OF HEALTH STATUS BETWEEN TWO GROUPS OF PATIENTS (x±s)

Subsets	Before treatment	After treatment	t value	p value	Before treatment	After treatment	t value	p value
	Health status				Physical pain			
Experimental group	22.30±5.12	44.09±11.50	-12.24	<0.001	27.10±5.60	45.66±10.16	-11.31	<0.001
Control group	21.86±5.64	32.76±8.49	-7.56	<0.001	26.66±6.05	31.56±8.53	-3.31	<0.001
t value	-0.41	-5.6			-0.38	-7.52		
p value	0.684	<0.001			0.707	<0.001		
Subsets	**Mental health**				**Energy**			
Experimental group	22.74±7.60	49.08±8.93	-15.88	<0.001	23.06±7.19	49.20±8.86	-16.2	<0.001
Control group	23.64±6.47	36.30±8.31	-8.5	<0.001	22.14±9.19	39.66±8.75	-9.76	<0.001
t value	0.64	-7.4			-0.7	-5.42		
p value	0.525	<0.001			0.488	<0.001		

Conflict of interests:

The authors declared no conflicts of interest.

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