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Research Article
Comparing Efficacy and Safety of Local and Systematic Zoledronic Acid in Autogenous Bone Grafts

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Abstract

Background and Objective: Resorption of the graft is a major problem in autogenous bone graft technique. The objective of the study was to evaluate efficacy and safety of local zoledronic acid in autogenous bone grafts technique. **Materials and Methods:** Three hundred ninety-eight patients had complex proximal humerus fracture surgery were randomized in five group each containing 62 patients. The anterior iliac crest bone was used as a graft. The grafts were placed in normal saline (group I), 1 mg mL^{-1} zoledronic acid for 10 s (group II), for 11 min (group III). All grafts were rinsed 4×4 min with normal saline. There were 1 mg/5 mL zoledronic acid pipetted to the grafts and were not rinsed (group IV). The graft was placed in normal saline and 4 mg/5 mL zoledronic acid was injected systemically (group V). All patients were evaluated for the range of the motion and the radiographic analysis during follow-up. The two-tailed paired t-test/Dunnett's multiple comparison tests were used at 95% of confidence level. **Results:** Group I ($p = 0.007$, $q = 2.58$) and group II ($p = 0.0038$, $q = 2.601$) had possessed significant recovery after 12 months of discharge. Group IV ($p = 0.0089$, $q = 3.425$), had possessed significant recovery after 6 months during follow-up. However, group III ($p = 0.0011$, $q = 5.202$) and group V ($p = 0.0019$, $q = 4.918$) had observed significant recovery after 3 months during follow-up. **Conclusion:** It was concluded that local treatment of zoledronic acid had a significant role in the recovery of autogenous bone grafts.

Key words: Autogenous bone grafts, biomechanical motion, complex proximal humerus fracture, local soaking, zoledronic acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The humerus is the bone fracture of the upper arm. In the condition where complex proximal humerus fracture (CPHF) surgery could be difficult, the bone graft (BG) is only available option for surgery[1]. The BG is one of the surgical methods that replace missing bone for repairing bone fractures that are too complex, pose a significant health risk to the patient, or fail to heal properly[2]. There is a new development in BG techniques. Autogenous bone grafts (ABGs) are the technique in which BG is taken place using patients' own bone[1]. The ABG is "Gold standard" regarding implantation[3]. The purpose of the ABG is vascularized and remodeled of the incorporated bone[4]. There is a need of newborn formation as well as healing of incorporated bone. There is a balance available between bone formation and bone resorption. However, due to the absence of blood circulation and chemical signals, the anabolic method is failed[5]. Hyperactivity and catabolic activity may lead to resorption of graft[4]. The systematic bisphosphonates have been used to manage the resorption of graft[6].

Local administration of bisphosphonates directly to graft is provided strong chemical binding to its phosphate group and the hydroxyapatite crystals of bone[7]. However, bisphosphonates may affect metabolism and osseointegration of the other normal bones[8]. Alendronate is bisphosphonate with good activity and lower affinity to the bone but high concentration is required for significant effect[9]. Zoledronic acid or Zoledronate (ZA) is the third-generation bisphosphonates with nitrogen[10] and has the strongest bone binding affinity[11].

The aim of this study was to evaluate efficacy and safety of local and systematic ZA for ABG in patients had CPHF.

MATERIALS AND METHODS

Materials: The ZA for Injection was purchased from Novartis Phama Schweiz AG Co., Ltd. Beijing, China. Normal saline (NS) was purchased from Chenxin Medical Supplies Co., Ltd., Shandong, China.

Ethical statement: The ethics committee for human experiments of The First Affiliated Hospital of Fujian Medical University, China approved the experimental protocol and the ethical guidelines for biomedical research on human participants in accordance with the Chinese law were followed[12].

Inclusion criteria: The single-center study was carried out between January, 2010 and December, 2016, The First Affiliated Hospital of Fujian Medical University, China treated more than 400 patients of CPHF surgery. Among them, 398 patients had CPHF due to a motor vehicle accident and industrial crashes with signed informed consent form were included in randomization as per Table 1. The neer classification of CPHF at baseline is shown in Table 2.

Exclusion criteria: Patients, who had refused to the informed consent form, refused to follow-up the study, renal failure and mineral metabolism disturbance excluded from the study.

Table 1: Age, sex and body mass index analysis of randomized patients

	Sample size						Age (year)					BMI (kg cm^{-2})				
	Male		Female		Total											
Groups	N	%	N	%	N	%	Mean	SD	SE	Min	Max	Mean	SD	SE	Min	Max
I	58	94	4	6	62	100	34.74	6.78	0.86	25	45	25.39	2.10	0.27	21	28
II	57	92	5	8	62	100	35.24	6.94	0.88	25	45	25.58	2.65	0.34	23	26
III	55	89	7	11	62	100	35.96	8.00	1.02	25	53	25.24	2.45	0.31	22	27
IV	51	82	11	18	62	100	35.74	7.40	0.94	25	45	24.73	3.63	0.46	23	28
V	53	85	9	15	62	100	37.18	7.78	0.99	25	54	24.92	2.19	0.28	21	27

SD: Standard deviation, SE: Standard error, Data were represented as Number (Percentage), BMI: Body mass index

Table 2: Fracture analysis as per Neer classification system

	Types of fracture					
Groups	I	II	III	IV	V	VI
I	4 (6)	42 (68)	5 (8)	6 (10)	3 (5)	2 (3)
II	7 (11)	38 (61)	8 (13)	5 (8)	3 (5)	1 (2)
III	7 (11)	36 (58)	6 (10)	5 (8)	7 (11)	1 (2)
IV	6 (10)	39 (63)	6 (10)	5 (8)	4 (6)	2 (3)
V	5 (8)	38 (61)	10 (16)	7 (11)	1 (2)	1 (2)

Data were represented as Number (Percentage), n = 62 for all groups

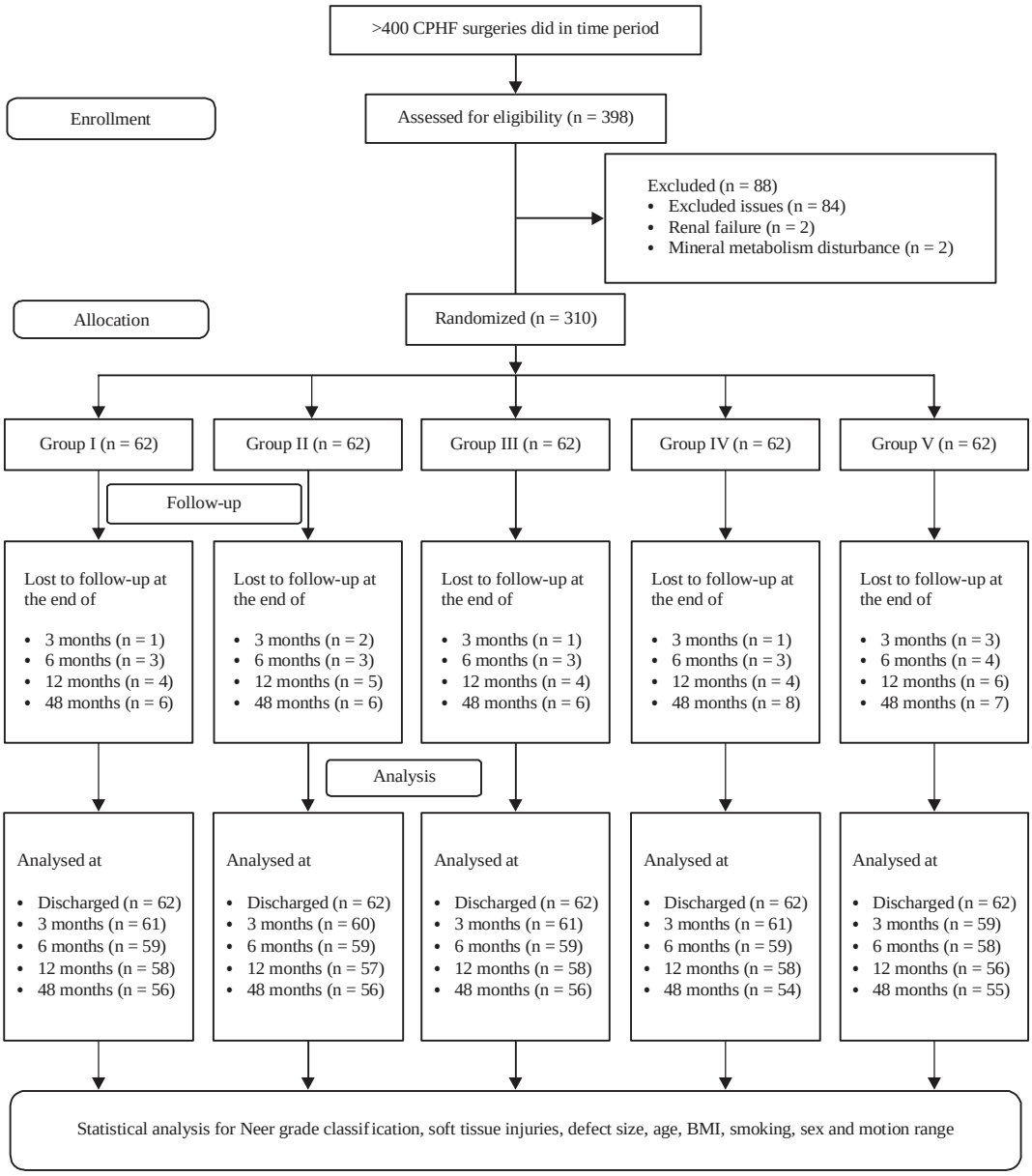

Fig. 1: Allocation, enrollment, follow-up and analysis of complex proximal humerus fractured patients used for autogenous bone grafts surgery

Prior sample size calculation: The prior sample size was calculated with Open Epi 3.0.1 (Epidemiologic Statistics for Public Health) the sample size was found to be 62 for each of the group. The parameters were considered for calculation of prior sample size were population size (N) = 398, confidence level = 95 %, percentage hypothesized frequency (p) = 95±5, z = 1.96, Design effect = 1. The five-arm study chart is shown in Fig. 1.

Surgical technique and grafts: Under ketamine anesthesia, patients were operated via a deltopectoral approach. Aiming to restore the missing bone and healing of the CPHF bone bed of the defect was augmented with the anterior iliac crest ABG (Fig. 2a)[13]. The fixation was carried out by titanium steel plate and screw (Xiame Double Import and Export Co., Ltd., Xiamen, China) (Fig. 2b). The compression screws were used in the anterior and posterior holes[14].

Intervention: The graft was kept sterile and stored in the aseptic area. The ZA lyophilized powder was dissolved in saline. Immediately before incorporation, each graft was

Table 3: Intervention of the study

Groups	Treatments		
	Drugs	Soaking time	Rinse in NS (times×min)
I (Negative control)	NS	None	4×4
II	ZA (1 mg mL^{-1})	10 sec	4×4
III	ZA (1 mg mL^{-1})	11 min	4×4
IV	1 mg/5 mL ZA topical admiration	None	None
V (Positive control)	4 mg/5 mL ZA systemic injection	None	4×4

NS: Normal saline, ZA: Zoledronic acid

Fig. 2(a-b): (a) Operative-condition for complex proximal humerus fracture and (b) Titanium steel

soaked in respective ZA solution[6]. The grafts were placed in NS (group I), 1 mg mL^{-1} ZA for 10 s (group II), 1 mg mL^{-1} ZA for 11 min (group III). All grafts were rinsed 4 times for 4 min in an aseptic area with NS to remove unbound ZA[8]. There were 1 mg/5 mL ZA pipetted to the grafts and were not rinsed (group IV). The graft was placed in NS and 4 mg/5 mL ZA was injected systemic (group V) (Table 3)[6]. The all patients were put on sitafloxacin for next four weeks of treatment[15].

Range of the motion: The patients were discharged 3 days after surgery. The self-directed rehabilitation with pendulum exercise started after 3 days of the surgery. The exercise did 4 times a day and 6 min each session. The individuals were motivated to use oneself hand for their daily activities. After 25 days of surgery, the patients also consulted a physiotherapist for exercises[14]. All patients were evaluated for the range of the motion at the end of 3, 6, 12 and 48 months[16].

Follow-up: Patients were evaluated for premature resorption of the graft, subsidence, infection, union, functional recovery and effect of smoking. Union was evaluated by the ability of an individual to bear full weight without pain and by radiographical analysis. The radiographic analysis was also carried out at the end of 3, 6, 12 and 48 month for fractured lines. All radiographical images were evaluated by the committee of three orthopedic trauma surgeons, one physician and one general surgeon. All were blind for the study[16].

Safety study: The patients were examined for acute toxicities such hypocalcemia, fever, bone pain, decreased oral intake, flu-like symptoms and myalgia at discharge time, at the end of 3 and 6 months[11]. The study was also evaluated for chronic inflammatory cells and necrosis at the end of 48 months[6].

Statistical analysis: The two-tailed paired t-test, GraphPad InStat, StateMate, GraphPad Software, Inc., USA (considering $\alpha = 0.05$ and $\beta = 0.1$ for significant difference)[16] followed by Dunnett's multiple comparison test, GraphPad InStat, StateMate, GraphPad Software, Inc., USA (considering $q \geq 2.457$ for significant difference)[17] was applied for Neer grade classification, soft tissue injuries, defect size, age, BMI (body mass index), smoking, sex and motion range. For Statistical analysis of motion range, movements without pain were considered as 1 and with pain considered as 0. All results were considered significant at 95% of confidence level.

RESULTS

There was an insignificant difference of Neer grade classification, soft tissue injuries and defect size between the groups at baseline ($p \geq 0.05$, $q \leq 2.457$).

There were 9 patients (1, 2, 2, 1 and 3 from the group I, II, III, IV and V, respectively) lost during first 3 months of follow-up after surgery for analysis. Further, 7 patients (2, 1, 1, 2 and 1 from the group I, II, III, IV and V, respectively) lost during the next 3 months of the follow-up period. Moreover, 8 patients (1, 2, 2, 1 and 2 from the group I, II, III, IV and V, respectively) lost during the next 6 months of the follow-up period. There were 12 patients (2, 2, 3, 4 and 1 from the group I, II, III, IV and V, respectively) lost during the next 36 months of the follow-up period.

There was the insignificant effect of age, BMR, smoking and sex on time of union and frequency of union ($p \geq 0.05$, $q \leq 2.457$ for all). Group I ($p = 0.007$, $q = 2.58$) and group II ($p = 0.0038$, $q = 2.601$) had possessed significant recovery after 12 months during follow-up. Group IV ($p = 0.0089$, $q = 3.425$), had possessed significant recovery after 6 months during follow-up. However, group III ($p = 0.0011$, $q = 5.202$) and group V ($p = 0.0019$, $q = 4.918$) had observed significant recovery after 3 months during follow-up. There was an insignificant improvement in motion range in group II and IV as compared to group I at discharge, at the end of 3, 6, 12 and 48 months during follow-up. However, there were a significant improvement in motion range in group III and V as compared to group I at discharge, at the end of 3, 6, 12 and 48 months during follow-up (Table 4).

The radiographic analysis showed that there was no dissolution of the graft after surgery and good union of the graft. None of the patients had loss of plate deformation (Fig. 3).

All patients had post-operative pain at the harvest site and were overcome after 15 months. There was no infection at the harvest site.

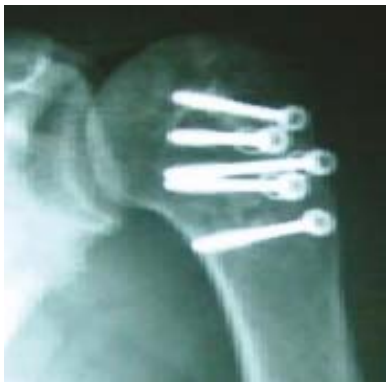

Fig. 3: Radiograph at the end of 3 months of follow-up

Table 4: Analysis of biomechanical motion study during follow-up time

		Motion range at the end of time (Months)				
Groups	Movement without pain	Discharge	3	6	12	48
	Total numbers of patient analyzed (N) (%)	62	61	59	58	56
I	45°	62 (100)	47 (77)	39 (66)	31 (53)	23 (41)
	90°	0 (0)	13 (21)	15 (25)	17 (29)	6 (11)
	135°	0 (0)	1 (2)	4 (7)	7 (12)	10 (18)
	175°	0 (0)	0 (0)	1 (2)	3 (6)	17 (30)
		62	60	59	57	56
II	45°	55 (89)	40 (67)	32 (54)	22 (39)	18 (32)
	90°	7 (11)	16 (27)	17 (29)	20 (35)	17 (30)
	135°	0 (0)	4 (6)	7 (12)	8 (14)	10 (18)
	175°	0 (0)	0 (0)	3 (5)	7 (12)	11 (20)
		62	60	59	57	52
III	45°	30 (48)	10 (17)	9 (15)	1 (2)	0 (0)
	90°	20 (32)	30 (50)	26 (44)	27 (47)	8 (15)
	135°	12 (20)	16 (27)	18 (31)	19 (33)	13 (25)
	175°	0 (0)	4 (6)	6 (10)	10 (18)	31 (60)
		62	61	59	58	54
IV	45°	54 (87)	44 (72)	24 (41)	24 (41)	18 (33)
	90°	8 (13)	12 (20)	14 (24)	13 (22)	15 (28)
	135°	0 (0)	5 (8)	10 (17)	10 (18)	12 (22)
	175°	0 (0)	0 (0)	11 (18)	11 (19)	9 (17)
		62	59	58	56	55
V	45°	52 (84)	42 (71)	34 (59)	22 (39)	8 (14)
	90°	10 (16)	12 (20)	15 (26)	13 (23)	25 (45)
	135°	0 (0)	5 (9)	7 (12)	10 (18)	12 (22)
	175°	0 (0)	0 (0)	2 (3)	11 (20)	10 (19)

Data were represented as Number (Percentage)

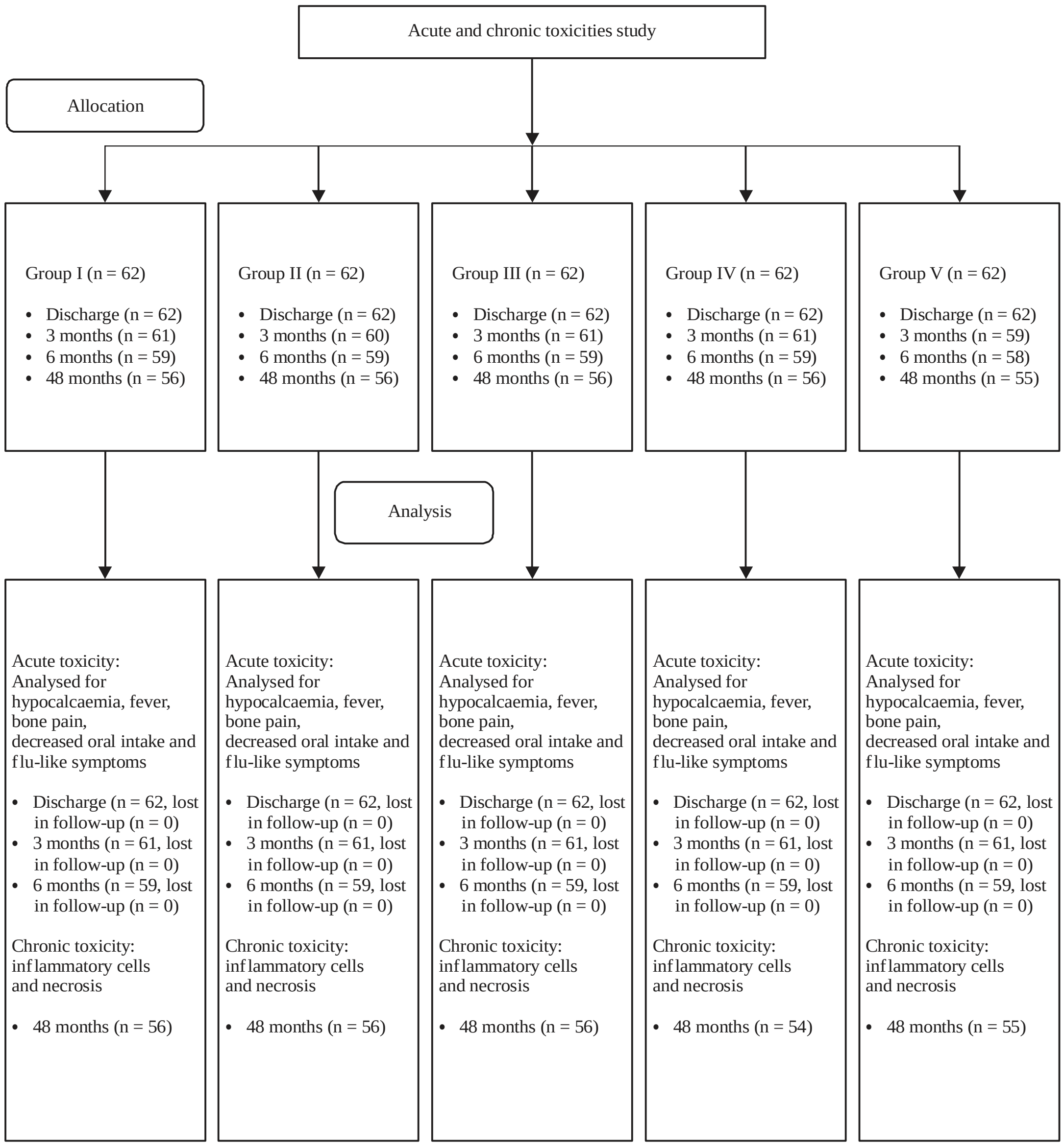

Fig. 4: Toxicities study flow-chart during follow-up

Table 5: Acute and chronic toxicities study of zoledronic acid after surgery

TT			Acute				Chronic	
TE			At DC				At 48 months	
Groups	Hypocalcaemia	Fever	Bone pain	Decreased oral intake	Flu-like symptoms	Myalgia	Inflammatory cells	Necrosis
I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
II	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
III	1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
IV	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
V	5 (8)	7 (11)	3 (5)	15 (24)	13 (21)	4 (6)	1 (2)	1 (2)

DC: Discharge time, TE: Time of evaluation, Data were represented as Number (Percentage), TT: Type of toxicities

The enrollment, analysis, evaluation of patients participated in safety study were shown in Fig. 4. There were significantly high acute toxicities in group V patients than any the other treatment ($p \leq 0.05$, $q \geq 2.457$ for all, Table 5).

DISCUSSION

In the current study missing bone deficit was overcome with the anterior iliac crest ABG. The anterior iliac crest autogenous bones are the gold standard for CPHF surgery[18]. In respect to the selection of bone for the graft, the surgery for the harvest of bone in the study was painful, secondary surgical procedure.

Grafts in all groups were rinsed 4 times for 4 min in an aseptic area with NS except group IV before surgery. The unbound ZA interferes with bone formation and a limited inhibition of ingrowth of the graft is seen[19]. In correlations to the medical technology adopted in the interventions, the study designed such that resorption and collapse of ABG were done immediately.

Group III and V had reported recovery after 3 months while group II had reported recovery after 6 months. Soaking time of the graft improves the ingrowth distance[6,18,20]. In respect to the results of the study, the finding was novel for union and the functional recovery of the grafts.

The biomechanical study reported that there was significant recovery and union of the graft in group III and V after 3 months as compared to the other treatments. The soaking of the graft in ZA for 11 min was provided the same effect to the ZA systematic injection ($p = 1.952$, $q = 0.9521$) without the acute toxic effects of ZA. The ZA has osteoconductive and osteoinductive properties[9,21]. In respect to the route of the administration of ZA selected for the intervention, the study was a totally novel concept in the field of orthopedic surgeries.

The study used ZA among the bisphosphonate. The ZA has comparatively and prolonged duration of action than the other bisphosphonates[22]. Alendronate and risedronate have high enzyme binding affinity which may lead to affect metabolism and osseointegration of the other normal bones[23]. Ibandronate is a stronger inhibitor of farnesyl pyrophosphate synthase (FPS) enzyme but weaker mineral binding affinity than ZA[24]. Tiludronate, clodronate and etidronate have no affinity for FPS enzyme, therefore, have low biochemical potential due to low mineral affinity[25]. With respect to the selection of bisphosphonate, the study considered being effective in CPHF.

The current study was on the five-arm human clinical trials and also evaluated biomechanical parameters and acute and chronic toxicities of ZA. Till date, an available study on effects of ZA in ABG surgeries was in rats[4,6] and goats[26] only. The available human study on ABG is not evaluated biomechanical studies[14,16]. Moreover, acute and chronic toxicities studies of ZA are not evaluated in the previous studies[11]. With respect to the subject selection and evaluation parameters of the study, the finding was more authentic than present research work.

Secondary and painful surgery was involved. There was too long follow-up period during the study.

CONCLUSION

The clinical study concluded that zoledronic acid had a significant role in the functional recovery of autogenous bone grafts in patients had complex proximal humerus fracture surgery. Local treatment and intravenous injection both had similar efficacy outcomes but intravenous injection had more common acute toxicities.

SIGNIFICANCE STATEMENT

This study discovers bone binding affinity of local zoledronic acid in a human subject that can be beneficial for autogenous bone grafts technique. This study will help the orthopedic surgeons to uncover the critical areas of bone grafting that many researchers were not able to explore.

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