Original Article

Results of type C fractures of distal radius treated by volar locking plate (VLP), an observational comparative study

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Received December 28, 2016; Accepted September 4, 2017; Epub October 15, 2017; Published October 30, 2017

Abstract: Purpose: This study aims to evaluate volar locking plate for treatment of type C fractures of the distal radius involving the lunate facet and compare them with those without involvement in lunate facet. Methods: A total of 67 patients with die-punch fractures of the distal radius were included and analyzed. Preoperative radiographs were used to diagnose and classify this injury. Radiographs taken at immediate postoperation and at last follow-up were analyzed. The main measurements were radial subsidence, articular step-off status, volar tilt, radial inclination, wrist motion range, grip strength, DASH score and related complications. Results: At the median time of 8 weeks, bony union was reached in all participants. At the final follow-up, there were no significant difference observed in term of volar tilt and radial inclination between both groups (P, 0.732; 0.644). The radial subsidence in DP group and articular step-off was significantly greater than that of NDP group. Articular step-off >2 mm occurred in 5 patients (23.8%) in DP group and 2 (4.3%) in NDP group (P=0.027). At 3-months after surgery, patients in DP group performed significantly worse than NDP group for most variables (P<0.05). At 6-months after surgery, the significance for wrist flexion, supination, ulnar deviation and pain perception remained. At the last follow-up, no significant differences were observed for any variable. Several minor complications occurred in either group, but with non-significance (P>0.05). Conclusions: Compared to non-die-punch fractures, patients with die-punch fractures would suffer more slowly recovery in term of wrist motion and pain perception, in addition to the increased risk of loss of reduction and final articular step-off (23.8%). In addition, the potential future radial-carpal osteoarthritis in the next few years should not be ignored, especially in those with significant articular step-off.

Keywords: Die-punch fracture, lunate facet, distal radius, comparative, volar locking plate

Introduction

Fractures of the radius or ulnas are the most commonly seen fracture type in emergency department and account for approximately 1/6 of fractures in adults, with 41-50% involving distal articular surfaces [1-3]. Generally by conservative treatments, patients with extra-articular or simple intra-articular fractures could obtain favorable clinical and radiographic results. However, for unstable fractures with intra-articular comminution and displacement, it could be impossible in restoration of anatomical alignment and articular congruity by non-operative methods. Therefore, for these complex intra-articular fractures, open reduction and internal fixation has been consistently regarded as the popularized treatment choice [4-6].

In 1962 year, Scheck et al initially proposed die-punch fracture, and regarded it as the dorso-medial fragments separated from the lunate facet [7]. Afterwards, the definition of die-punch fracture was extended and indicated distal radius fractures that involved lunate face. This type of injury commonly occurs in younger patients when they fall from height or suffer from vehicle accidents. And the subsequent results
are lunate facet collapse and fragments impact proximally into the radial metaphysics, commonly accompanied by significant displacement in different directions. According to the AO/ASIF classification system, Die-punch fracture was classified as AO type 23B1, 23B3 and 23C1-3.

Die-punch fracture represents a greater challenge for orthopaedics surgeons. Earp and his colleagues investigated outcomes of a series of AO type C3 distal radius fractures treated by a single volar plate and found that, most cases (5/8) of postoperative significant reduction loss occurred in those with initial lunate facet involvement [8]. Likewise, Rozental et al reported in a study of 41 dorsally displaced unstable fractures of the distal radius that, 50% (2/4) of cases of reduction loss occurred in those with lunate facet involvement [9]. Similar as the most other intra-articular distal radius fractures, the aim of treatment of die-punch fracture is to restore anatomical alignment and articular congruity, which closely relates functional results. Conversely, modification of the joint congruity would result in decreased motion range and increased symptomatic arthrosis, which significantly affect patients’ daily activities [10, 11].

So far, there is scarce of reported data on results for die-punch fractures, partially due to its infrequency [12-14]. In our opinion, it is very necessary to understand the characteristics and its prognostic information of this specific fracture type, not only because of its special mechanism but more of high technical demanding and difficulty in fracture reduction and fixation procedure. Given that, we performed this retrospective study to compare clinical results of AO type C fractures with lunate facet involvement with those without lunate facet involvement. The outcome parameters were radial subsidence during fracture healing period, volar tilt, radial inclination, wrist motion range, grip strength, articular step-off, and disabilities of the arm, shoulder, and hand (DASH) at the last follow-up visit; and related complication during the whole follow-up period.

Materials and methods

This study was approved by institutional review board of our institution. The study was designed as retrospective and we inquired the register database for rude diagnosis of distal radius fractures (764 cases) from 2013 January to 2014 December. Then, Picture Archiving and Communication System (PACS) were inquired for X-radiographs of initial injury for definite diagnosis and further classification according to AO comprehensive classification system. This step was accomplished by two trained investigators, and consulted a third investigator (experienced radiologist) for final decision if they have any disagreement on fracture classification.

Inclusion and exclusion criteria

Inclusion criteria were age of 18 and 60 years; definitely diagnosed type C fracture of distal radius by preoperative radiographs, CT scanning and reconstruction if necessary; no history of fracture or arthropathy at the injured hand; unilateral fracture; no concomitant injury in the injured upper extremity; fractures treated by volar locking plate (VLP) with or without auxiliary devices; complete follow-up data >12 months available. Exclusion criteria were old fracture (>2 weeks), history of operation at the injured forearm and wrist; open fracture with damaged soft tissue; lost to follow up or inadequate follow up data.

After screened by the aforementioned inclusion and exclusion criteria, a total of 67 patients with type C fractures treated by VLP were included, with 21 in die-punch group (DP group, with involvement of lunate facet) and 46 in non-die-punch group (NDP group, without involvement of lunate facet). Of them, 45 were males and 22 were females and their median age was 41 years (range, 18-63 y). The right wrist was involved in 43 patients and the left in 24; 49 involved the dominant hand and 18 involved in the non-dominant hand. According to the AO/AFIF classification system, AO type 23-C3 (41 cases) were the most common type, followed by C2 (16) and C1 (10). Accidents from electric bicycles were the most common cause, accounting for 43.2% (26/67) of all the injuries, followed by fall from height (17 cases), accidents from motor vehicles (11 cases), sports injuries (4 cases) and others (9 cases). 13 patients have concomitant injuries including ulnar styloid fracture, ankle sprain, rib fracture and tibial and fibula fracture. These patients were all treated by open reduction and fixed with locking plate through the volar approach,
VLP for treatment of die-punch fracture of the distal radius

Table 1. Comparison of preoperative variables between DP and NDP groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>DP group (21 cases), mean (sd) or n (%)</th>
<th>NDP group (46 cases), mean (sd) or n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.6 (14.2)</td>
<td>41.4 (16.1)</td>
<td>0.637</td>
</tr>
<tr>
<td>Males</td>
<td>15 (71.4)</td>
<td>30 (65.2)</td>
<td>0.616</td>
</tr>
<tr>
<td>Involvement side (right)</td>
<td>13 (61.9)</td>
<td>30 (65.2)</td>
<td>0.793</td>
</tr>
<tr>
<td>Handedness side</td>
<td>15 (71.4)</td>
<td>34 (73.9)</td>
<td>0.831</td>
</tr>
<tr>
<td>AO type</td>
<td></td>
<td></td>
<td>0.169</td>
</tr>
<tr>
<td>C1</td>
<td>3 (14.3)</td>
<td>7 (15.2)</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>2 (9.5)</td>
<td>14 (30.4)</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>16 (76.2)</td>
<td>25 (54.4)</td>
<td></td>
</tr>
<tr>
<td>Mechanism</td>
<td></td>
<td></td>
<td>0.335</td>
</tr>
<tr>
<td>Electric bicycles accidents</td>
<td>9 (42.9)</td>
<td>17 (37.0)</td>
<td></td>
</tr>
<tr>
<td>Falls from height</td>
<td>7 (33.3)</td>
<td>10 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Motor vehicles</td>
<td>4 (19.0)</td>
<td>15 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Sport</td>
<td>0</td>
<td>4 (8.7)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1 (4.8)</td>
<td>8 (17.4)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Comparison of preoperative variables between DP and NDP groups

at the mean interval of 5.5 days (1-14 days) between initial injury and surgery. No significant difference was investigated between groups (die-punch and non-die-punch fractures) for any preoperative variable; the results were presented in Table 1.

Surgery techniques

With the use of tourniquet, we made the traditional Henry approach and extended it distally 2 cm if necessary but without crossing the wrist crease for adequate exposure of fracture fragment. After retracting flexor carpi radialis, medial nerve medially and brachioradialis and radial artery and vein laterally, the pronator quadrates was exposed and incised from proximity of its radial insertion, with 1-2 cm preserved for suture. Then, retract the pronator quadrates ulnar, fracture site is exposed; fracture fragments are identified and reduced under fluoroscopic guidance. For classical Die-punch fracture that fracture line at the plane of impaction is transverse, periosteum elevator is introduced into the transverse impaction line to elevate the fragments until obtaining eveness of the subchondral bone line of the lunate facet with that of the scaphoid facet. For the other non-classical type of die-punch fracture that fracture fragments are impacted longitudinally and separated from the lunate facet, periosteum elevator is introduced into the longitudinal fracture line, disimpact the fracture fragments and then push them radially in line with the scaphoid facet. Temporary K wires were used to stabilize the reduced fragments. For cases of severely comminuted intra-articular or metaphyseal fractures where a large defect is created after reduction, or small lunate facet fragment that is unable to fix by screws, autologous iliac crest bone grafts are used to fill the gap and buttress the reduced fragments. For dorsally displaced lunate facet fragment, sometimes auxiliary kirschner wires or external fixator was used to stabilize the fractures. Under the control of fluoroscopy, A T-shape locking plate (Synthes™, Shanghai, China; Wego™, Shandong, China) is then applied on the volar side and the sliding hole is fixed first which allows readjustment according to status of articular surface. Distal screws are placed just beneath the subchondral bone, which provides the maximum ability to buttress the lunate facet fragments. Temporary K wires were removed and the proximal locking screws were then placed. Re-confirm the placement of VLP radiographically and particular care is taken to prevent the distal locking penetrating beyond the dorsal cortex. After desired reduction and fixation, the pronator quadratus is repaired with absorbable suture material and a dressing was placed (Figure 1). Postoperatively, remove the drape at 2-4 days based on exudation status. Other than severely comminuted fracture with potential secondary displacement (type C3.3), all wrists were not placed any external fixation device (plaster cast or splint). Active finger motion was started the day after the surgery. Dressings and sutures were removed in the fourteenth postoperative day. Patients were encouraged to start wrist joint when evidence for bony cal-    

Satisfactory reduction was defined as dorsal tilt less than 10°, volar tilt <20, radial inclination >10, radial shortening <2 mm and articular step-off <1 mm [15, 16], at the immediate postoperative X-rays. And satisfactory reduction
was obtained in all patients. Postoperatively, patients were followed up routinely at 2 weeks, 1 month, 3 months, 6 months and 1 year postoperatively. Posteroanterior (PA) and lateral X-rays taken at immediate postoperation and at follow-ups were used to evaluate radiographic parameters. We defined radius subsidence as the absolute difference value between both ulnar variances measured at X-rays of immediate postoperation and patients’ last follow-up. Articular step-off was evaluated from the last-visit X-rays, with the value of 2 mm or greater defined as significant one [17]. Likewise at the last follow-up visit, grip strength wrist motion range including flexion, extension, pronation and supination were measured and compared to the contralateral normal side using percentage.

Functional outcome was evaluated by Disabilities of the Arm, Shoulder and Hand (DASH), which emphasizes patients’ ability to perform daily activities with score ranging from 0 to 100. The higher scores represented the poorer functional result, with 0 points representing no disability and 100 points representing maximum disability. Pain in wrist motion and at rest was evaluated by visual analogue scale, with 0 representing no pain and 10 representing maximum pain.

Data on bone and soft tissues-related complications during follow-ups were documented. The potential related complications included infection, tendon tenosynovitis, tendon contractions, carpal tunnel syndrome (CTS), implant failure (plate fracture or screw loosening), delayed union, non-union and symptomatic traumatic arthritis. After completion of routine follow-up (1 year), latter-period complications of which patients complained were also documented for evaluation.

**Statistical analysis**

Patients’ characteristics including age, volar tilt, radial inclination, radial subsidence, articular step-off, grip strength, range of motion, VAS and DASH were reported as continuous variables and expressed in mean and standard deviation (SD). Status of normality distribution of these variables was explored and confirmed. Any variable between both groups was compared using the Student’s *t*-test or Whitney-U
Table 2. Comparison of two groups for clinical and radiographic results at 3, 6 and last follow-up (>12 months)

<table>
<thead>
<tr>
<th></th>
<th>Three months</th>
<th></th>
<th>Six months</th>
<th></th>
<th>Last follow-up (&gt;12 months)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DP group</td>
<td>NDP group</td>
<td>P</td>
<td>Mean (sd)</td>
<td>% of Value on Contralat. Side</td>
<td>Mean (sd)</td>
</tr>
<tr>
<td>Flexion (deg)</td>
<td>33 (17.2)</td>
<td>51.7</td>
<td>0.001</td>
<td>47.6 (12.1)</td>
<td>74.8</td>
<td>53.9 (11.6)</td>
</tr>
<tr>
<td>Extension (deg)</td>
<td>37 (16.6)</td>
<td>55.8</td>
<td>0.004</td>
<td>57 (12.7)</td>
<td>86.4</td>
<td>60.2 (12.1)</td>
</tr>
<tr>
<td>Pronation (deg)</td>
<td>56.5 (12.8)</td>
<td>66.8</td>
<td>0.023</td>
<td>74.2 (7.7)</td>
<td>85</td>
<td>76.8 (8.9)</td>
</tr>
<tr>
<td>Supination (deg)</td>
<td>58 (10.8)</td>
<td>59.3</td>
<td>0.007</td>
<td>73 (8.8)</td>
<td>76</td>
<td>78.8 (9.4)</td>
</tr>
<tr>
<td>Radial deviation (deg)</td>
<td>11 (6.3)</td>
<td>58.8</td>
<td>0.218</td>
<td>17.2 (4.9)</td>
<td>83.4</td>
<td>18.4 (6.2)</td>
</tr>
<tr>
<td>Ulnar deviation (deg)</td>
<td>18.8 (8.5)</td>
<td>51.4</td>
<td>&lt;0.001</td>
<td>27 (6.3)</td>
<td>72.5</td>
<td>32.6 (7.8)</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>9.5 (5.5)</td>
<td>0.34.4</td>
<td>0.072</td>
<td>22.3 (6.8)</td>
<td>82.2</td>
<td>23.7 (9.1)</td>
</tr>
<tr>
<td>VAS at rest</td>
<td>2.5 (2.9)</td>
<td>2.1 (2.4)</td>
<td>0.030</td>
<td>2.3 (2.6)</td>
<td>1.9 (2.1)</td>
<td>0.041</td>
</tr>
<tr>
<td>VAS in motion</td>
<td>3.5 (3.2)</td>
<td>2.9 (3.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DASH score</td>
<td></td>
<td></td>
<td></td>
<td>15.6 (14.0)</td>
<td>13.2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Radial subsidence (mm)</td>
<td>1.1 (0.6)</td>
<td></td>
<td></td>
<td>0.6 (0.9)</td>
<td>0.2 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Articular step-off</td>
<td></td>
<td></td>
<td></td>
<td>7 (8.5)</td>
<td>8.1 (8)</td>
<td></td>
</tr>
<tr>
<td>Volar tilt</td>
<td></td>
<td></td>
<td></td>
<td>22 (5.5)</td>
<td>20.6 (6.2)</td>
<td></td>
</tr>
</tbody>
</table>
test according its normality status. Gender distribution and frequencies of complications were recorded as categorical variables and expressed in percentage. The Pearson chi-square test or Fisher’s exact test were used to analyze data of categorical variables. The threshold for significance was set at $P=0.05$. All analyses were performed using the SPSS 19.0 statistical software package for Windows 2007 (SPSS, Inc., Chicago, Illinois).

**Results**

**Radiographic results**

The median follow-up time was 14 months (range, 12-37 months). At the median time of 8 weeks (6-16 weeks), bony union was reached in all participants. At the final follow-up (12 months, minimum), the mean volar tilt was $7^\circ$ (sd, $8.5^\circ$) and radial inclination was $22^\circ$ (sd, $5.5^\circ$) in DP group, which did not differ from that of NDP group ($P=0.732; 0.644$). The radial subsidence in DP group was significantly greater than that of NDP group ($1.1\text{ mm vs }0.7\text{ mm}$). And the articular step-off was $0.6\text{ mm (sd, 0.9 mm)}$, which was significantly greater than the value of $0.2\text{ mm (sd, 0.4 mm)}$ of NDP group ($P=0.002$). Articular step-off $>2\text{ mm}$ occurred in 5 patients (23.8%) in DP group and 2 (4.3%) in NDP group, which was significantly different ($P=0.027$) (Table 2).

**Functional results**

At 3-months after surgery, patients in DP group performed significantly worse than NDP group for most variables of ROM, including wrist flexion, extension, pronation, supination and ulnar deviation ($P<0.05$). But there was no significant difference investigated for radial deviation (11 vs 13.4) ($P=0.218$). Compared with the contralateral uninjured wrist, patients regained wrist motion of 51.4-66.8% in DP group and 60.7%-71.2% in NDP group, respectively. The mean grip strength in the injured limb was 9.5 kg (34.4% of the contralateral uninjured limb) and 11.2 kg (35.1% of the contralateral uninjured limb) in the DP and NDP group, respectively ($P=0.722$). Patients in DP group had a greater VAS score at wrist motion (mean, 3.5; sd, 3.2) than that of NDP group (mean, 2.9; sd, 3.2) ($P=0.03$). But there was no significant difference observed for VAS score at rest ($P=0.072$) (Table 2).

At 6-months after surgery, the significance for wrist extension and pronation disappeared, and the difference for wrist flexion, Supination, Ulnar deviation and pain perception (VAS) remained significant. However, at the lost follow-up (12 months, minimum), no significant differences were observed for any variable ($P>0.05$) (Table 2).

**Complications**

Superficial infections occurred in 2 patients of DP group and 4 of NDP group, and were all resolved by a single course of oral antibiotics. Temporary median nerve symptoms developed in 1 patient of DP group and 3 of NDP group at early postoperative period. The symptoms persisted several weeks and gradually disappeared without intentional intervention. 2 patients of NDP group complained of persistent regional wrist due to prominent hardware, and obtained relief after hardware removal before scheduled period. There were no cases of re-fracture after plate removal, subjective digital stiffness, flexor tendon tendinitis and rupture during the follow-up period.

**Discussions**

Die-punch fracture of distal radius is a specific unstable fracture that was characterized by compressed lunate facet fragments, representing a great challenge for orthopaedic surgeons. So far as we know, there is scarce of reported data about the clinical results of die-punch fractures or comparative results between them with other types of fractures. The results in this study demonstrated VLP with or without auxiliary fixation could not provide adequate stability for all die-punch fractures, with resultant 23.8% of them developing significant articular step-off ($>2\text{ mm}$). Compared to the distal radius type C fractures without involvement in lunate facet, die-punch fractures resulted in worse clinical outcomes at early postoperative period (3- and 6 months). But the significance disappeared at the last visit (12 month, minimum).

From the view of anatomy, lunate facet accounted for 51.7%-53.0% of the whole distal radius articular surface [18-20]. And from the view of 3-column theory, lunate facet constitutes the intermediate column and is the most predominant elements of articular surface for load bearing. Therefore, how to effectively reduce
the impacted lunate facet fragments and keeping adequate stabilization is the key issue that deserves surgeons’ greatest attention. In this study, we applied the traditional Henry or extended approach so as to obtain maximum exposure of the articular surface to capture and fix the lunate facet fragments. For fractures with bone loss after fracture reduction in fractures with intra-articular multifragments and metaphyseal comminution, we recommend autologous iliac bone to fill the gap or buttress the small impacted luante facet fragments. In addition, for lunate facet fragments of dorsal displacement that were unable to capture and fix by single VLP, we applied auxiliary kirschner wires or external fixator to stabilize the fragments. Despite this, the mean values of articular step-off was 0.6 mm (sd, 0.9 mm) and there were 5 cases (23.8%) of significant articular step-off (>2 mm) found at the last follow-up, both of which were higher that of previous studies [13, 21, 22]. Primarily, we attributed this deviation to the greater severity of fractures, with C3 accounting for 76.2% in the DP group in this study. In contrast, the sample in their studies was type B3 or type B and C [22]. Therefore, multifragmentary fractures with impacted lunate facet significantly increased the difficulty in reduction and fixation during surgery. In addition, the specific anatomy of volar-ulnar corner of lunate facet made it impossible for VLP to provide similarly adequate stability for scaphoid and lunate facet [23-25]. Therefore, improvement of technical skills for fixation of small lunate facet fragments and innovative design of implants might be the future research subjects.

From 3- to 12-month follow-up postoperatively, the wrist motion of patients were improved gradually both in DP and NDP group. At 3-month postoperatively, DP groups performed significantly worse than NDP groups in most variables except for radial deviation, grip strength and VAS score at rest. At 6-month postoperatively, the significance of difference in wrist flexion, supination and ulnar deviation persisted, and disappeared at the last follow-up (>12 months). In our opinion, these results could be predictable and exactly reflected the important role of lunate facet playing in the wrist motion and function. Lunate facet was the cornerstone of joint motion and any wrist motion with lunate facet as rotation axis would be affected more or less. In contrast, motion like radial deviation was not affected so much as other variables, resulting in non-significance between two groups at any time points.

In the study of investigating results of AO type C3 fractures of distal radius, Schneeberger et al found favorable results in wrist motion and grip strength at postoperative 12 months (70-100% of the contralateral uninjured wrist) and 16/18 cases had none or mild pain [26]. In a study of AO type C3 fractures treated by dorsal or volar plate, Rein et al reported the better flexion in VLP group but better extension and radial deviation in dorsal plate group, providing us with an alternative for treating fractures with significant dorsal displacement and comminution [10]. Souer et al concluded in their study that, there were no significant differences between AO type-B and type-C volar shear fractures in term of motion, grip strength and DASH score at any time [27]. A direct comparison with results from these studies seems impossible, predominantly due to non-homogeneity of participants, in addition to lack of standardized and reproducible methods for evaluation. Therefore, we applied widely used VAS score and DASH core for upper extremity disabilities and the results were comparable to those in other studies [10, 26-28]. There was no significant difference between 2 groups for postoperative complications (intra-articular step-off except), which might was attributed to the similar preoperative variables (fracture type, age, gender, and mechanism et al), surgical approach and implants.

This study suffered from several limitations. Firstly, this nature of retrospective design might lead to recall bias, causing type II statistical error. For some patient-reported complications such as finger stiffness that resolved before long after exercises would not be documented. Secondly, most diagnoses and AO classification were performed based on radiographs, which might underestimate the severity of such injury. However, because we only included type C fractures, the odd of mis-and missed diagnose was should be very low, compared to partial intra-articular fractures of distal radius. Thirdly, the short follow up prevented the detection of radiocarpal osteoarthritis, especially in those with significant articular step-off (>2 mm). Therefore, careful subsequent follow-up of these patients were very necessary.
In this study, VLP for treatment of AO type C fractures of distal radius obtained favorable results at the final follow-up. Compared to non-die-punch fractures, patients with die-punch fractures would suffer more slow recovery in term of wrist motion and pain perception, in addition to the increased risk of loss of reduction and final articular step-off (23.8%). Although with similar functional results between both groups at 12 months, the potential future radialcarpal osteoarthritis in the next few years should not be ignored, especially in those with significant articular step-off.

Acknowledgements

We are grateful to X Zhang of the Department of statistics and applications, and to L Wang and D Zhang of the Department of Orthopedics for their kind assistance.

Disclosure of conflict of interest

None.

Authors’ contributions

Yong Shen and Yingze Zhang designed the study; Yan Zhao and Qiusheng Wang inquired the PACS, followed up the participants and documented the data; Wenyuan Ding and Qiusheng Wang analyzed and interpreted the data; Zhenmu Lv and Yan Zhao wrote the manuscript and Yong Shen approved the final version of the manuscript.

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