

Feasibility and potential of intraoperative ultrasound in arthroscopy of femoroacetabular impingement

Zhuohua Lin¹, Ligang Cui^{1,†}, Yan Xu^{2*,†}, Qiang Fu¹ and Youjing Sun¹

¹Department of Ultrasound, Peking University Third Hospital, 49 Huayuan N Rd, Haidian District, Beijing 100191, China and ²Department of Sports Medicine, Peking University Third Hospital, Institute of Sports Medicine of Peking University, Beijing Key Laboratory of Sports Injuries, 49 North Garden Road, Beijing 100191, China.

[†]Ligang Cui and Yan Xu have equal contributions to these article.

*Correspondence to: Yan Xu. E-mail: yanxu@139.com

ABSTRACT

This study aimed to evaluate the feasibility of using ultrasound for monitoring osteochondroplasty intraoperatively, determine the factors that interfere with ultrasound imaging and assess its influence on surgeon performance.

Intraoperative ultrasonography was performed during osteochondroplasty in 39 cases of arthroscopy. The femoral head–neck junction (FHNJ) was evaluated using ultrasonography. Another 39 cases, which underwent conventional arthroscopy, were included in the control group. The C-arm was used in this group at the end of osteochondroplasty to confirm that no residual cam lesion remained. Pre- and postoperative Dunn radiographs and computed tomography (CT) scans were analyzed to determine the feasibility of ultrasound.

Residual cam deformity was noted in eight cases under ultrasound. The FHNJ was not detected owing to ultrasound interference by air in three cases. No difference in the α angle measured in Dunn radiographs and the residual cam deformity rate of CT at the 1:00, 2:00 or 3:00 position was found between both groups. However, the α angle at the 3:00 position was greater in the ultrasound group than in the control group ($44.4 \pm 4.3^\circ$ versus $41.3 \pm 5.1^\circ$, $P < 0.05$), suggesting that more bone was preserved at 3:00 in the ultrasound group. No difference was found in the mean operation time. None of the patients experienced complications, such as wounds or intra-articular infections.

Ultrasound is a safe method for assessing FHNJ during arthroscopy. It did not change the risk of residual cam deformity but positively affected the surgeon's performance by reducing unnecessary bone removal.

INTRODUCTION

Hip arthroscopy has shown good results in treating femoroacetabular impingement (FAI). Arthroscopy tends to show earlier improvement, quicker recovery and faster return to sports than open surgery [1]. Osteochondroplasty is an important step during arthroscopy, as it removes cam deformity to restore concavity at the femoral head–neck junction (FHNJ).

Approximately 9.6% of patients after hip arthroscopy may require revision surgery or subsequent total hip arthroplasty [2]. Residual cam deformity at the FHNJ is the main reason for surgery failure [3]. However, increasing the depth, length and width of resection will increase the risk of fracture [4]. Thus, it is important to remove all the cam deformity and prevent over-resection.

Fluoroscopy using the C-arm is the most common tool used during osteochondroplasty to confirm the absence of residual cam deformity; however, residual cam deformity may still occur [5]. Compared to fluoroscopy, ultrasound imaging is a more convenient tool, producing no ionizing radiation. Moreover, it

provides real-time imaging. Several studies have reported its use in assessing the morphology of the FHNJ before and after arthroscopy [6–9].

This study focused on the feasibility of intraoperative ultrasound in hip arthroscopy, the factors interfering with ultrasound image quality, and its influence on surgeon performance, aiming to determine the feasibility of ultrasound in arthroscopy.

MATERIALS AND METHODS

Study design and participants

This study was approved by the Ethics Committee of our institution. Patients diagnosed with hip impingement between May 2019 and May 2020 who required arthroscopy at Peking University Third Hospital were recruited. The procedures followed the ethical standards of the Declaration of Helsinki (1964, amended most recently in 2008) of the World Medical Association.

Patients were included if they were at least 18 years old, reported aggravated or recurrent pain and limited hip mobility, had radiographic features of cam morphology, showed injury

or tear of the labrum on magnetic resonance imaging and received ineffective conservative treatment [1, 10]. Patients were excluded if they had a history of hip surgery or other hip joint diseases, including dysplasia, avascular necrosis of the femoral head, trauma or infection on the same side.

Eighty patients were included in this study. One patient was excluded because of hip dysplasia, and one patient was excluded because of a history of hip surgery. The remaining 78 patients were randomly divided into two groups. We performed intraoperative ultrasound in 39 patients. Another 39 patients underwent conventional surgery using intraoperative fluoroscopy in the control group. Informed consent was obtained from all patients.

Procedures

Arthroscopy for FAI was performed by a surgeon with 3 years of experience in hip arthroscopy and more than 200 arthroscopies per year. General or epidural anesthesia was administered, and the patient was placed in the supine position.

A Mindray color Doppler ultrasound system M9 (Mindray, Shenzhen, China), equipped with a C5-1S convex array probe and an L14-4s linear array probe, was used in this study. A musculoskeletal ultrasound doctor with 3 years of experience performed the pre-scanning and intraoperative ultrasound. A sterile plastic cover was wrapped around the probe during the surgery. Preoperative ultrasonography was performed to evaluate the range of cam deformities. Using the acetabulum clock-face location method, the indirect head, direct head of the rectus femoris and iliopsoas muscle tendon were identified, and then these tendons crossing the acetabular labrum positions were defined as 0:00, 2:00 and 3:00, respectively (Fig. 1) [11]. These locations were marked on the skin of the hip to help indicate the locations intraoperatively.

Preoperatively, hip traction was applied to allow the entrance of the arthroscope. Real-time ultrasonography was performed with a probe along the longitudinal section of the femoral neck [12]. Hip traction was considered complete when ultrasound imaging showed joint space broadening, which was reconfirmed by fluoroscopy. Anterolateral and anterior portals were established in the central and peripheral compartments of the hip under radiographic guidance, according to the surgeon's usual practice. The surgeon first repaired the acetabular labrum, released the traction and examined the surrounding compartments using arthroscopy. The cam deformity was removed using a burr.

In the ultrasound group, ultrasound assessment was performed whenever the surgeon requested it. Under ultrasonography, the presence of focal bony protuberances of the femoral neck, a distinctly irregular contour of the FHNJ or a non-spherical head-neck junction was defined as a residual cam deformity [6]. If a residual cam deformity was found via ultrasound, based on the preoperative mark, we determined its location based on whether it was near the anterior or anterosuperior femoral head. A caliper function was also used to locate the residual cam deformity and measure the distance between the residual cam deformity and the margin of the acetabulum. The surgeon further resected the cam lesion according to the position indicated by the ultrasound until no residual cam deformity

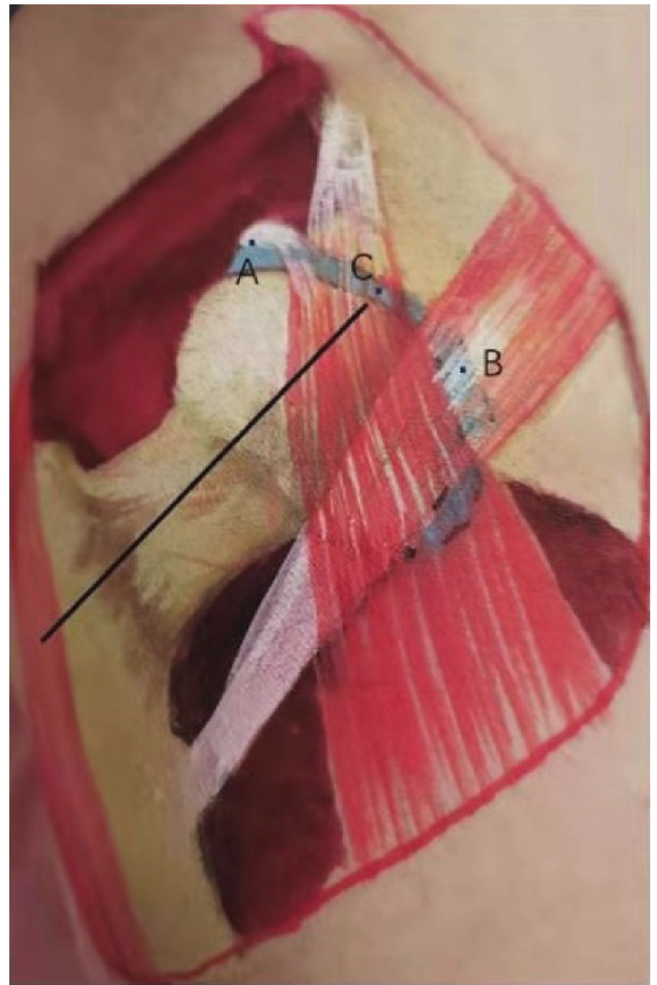


Fig. 1. Diagram of the hip joint. The indirect head (A), direct head of the rectus femoris (C) and iliopsoas muscle tendon (B) crossing the acetabular labrum represent the 0:00, 2:00 and 3:00 positions of the labrum, respectively.

was found. In the control group, fluoroscopy was performed in the anterior and 45° Dunn positions to confirm the absence of a residual cam deformity. The operation time and postoperative wound healing conditions were recorded.

Image analysis

Routine X-rays in the Dunn views and computed tomography (CT) scans were obtained pre- and postoperatively for all patients [13, 14]. The CT images were reconstructed by an experienced radiologist at 1:00, 2:00 and 3:00 using Mimics version 20.0. The radiologist was blinded to the intraoperative imaging method used to evaluate the cam deformity (Fig. 2) [15, 16]. The α angle was measured three times by the same radiologist and averaged. An α angle of $>55^\circ$ indicated the existence of cam deformity [17, 18].

Statistical analysis

The data were analyzed using SPSS version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables are presented as numbers and percentages, while continuous variables

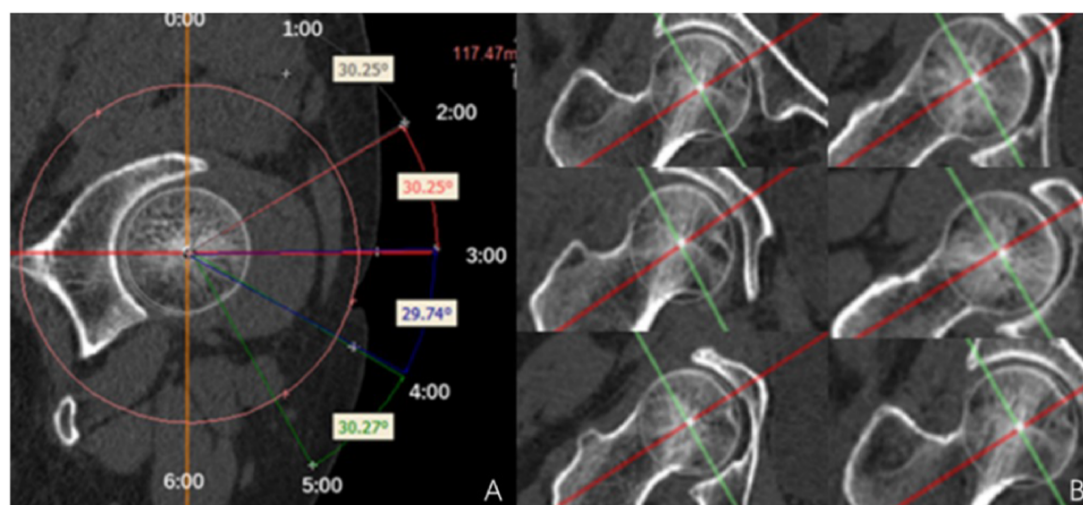


Fig. 2. Example of multiplanar reconstruction of a hip joint CT image. (A) The hip CT images were reconstructed based on plane A. (B) Different hip planes were acquired. The α angle of 1:00, 2:00 and 3:00 positions was measured.

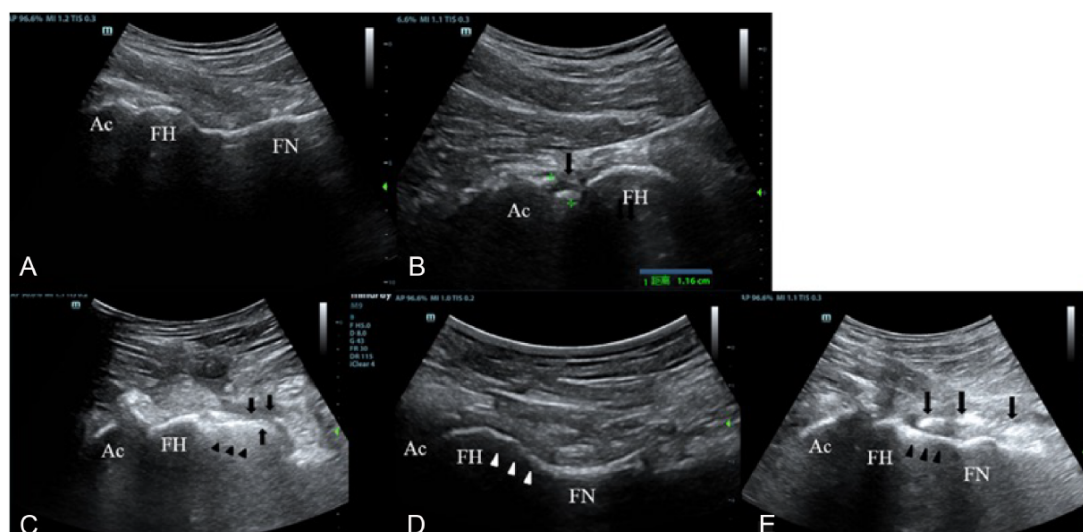


Fig. 3. Example of intraoperative ultrasound. (A) The acetabular and femoral head were in contact before traction. (B) After traction, the distance between the acetabular and femoral head increased, and an air-like hyper-echo (arrow) was found under the joint capsule. (C) Air that had accumulated inside the joint capsule interfered (arrow) with the observation of the FHNJ (arrowhead). (D) Preoperative ultrasound of the FHNJ shows asphericity (arrowhead) of the FHNJ, indicating the existence of FAI. (E) Intraoperative ultrasound showed the morphology change of the FHNJ (arrowhead). The cam deformity was removed, and an air-like hypo-echo (arrow) occurred inside the joint capsule.

Ac, acetabular; FN, femoral neck.

are expressed as the mean \pm standard deviation. A t-test was used to compare the α angles between the groups. The chi-squared test was used to compare the intergroup risk of cam deformities. Statistical significance was set at $P < 0.05$.

RESULTS

Intraoperative ultrasound findings

During traction, ultrasonography can show the distance between the acetabular and femoral heads in real time. With joint capsule distention, an air-like hyperechoic lesion was found under the joint capsule (Fig. 3A and B).

The FHNJ was not evaluated by ultrasound intraoperatively in three patients because the air in the joint blocked the sight

(Fig. 3C). Among the remaining 36 patients, a significant morphological change in the FHNJ was observed under ultrasound (Fig. 3D and E). Residual cam deformity was noted on ultrasonography intraoperatively in eight patients. Intraoperatively, a residual cam lesion was most likely to present as a bony protuberance at the FHNJ (Fig. 4).

Pre- and postoperative radiographs and CT images

The α angle of the remaining 36 patients in the ultrasound group and 39 patients in the control group were analyzed. There were no significant intergroup differences in the distribution of sex or symptom duration ($P > 0.05$; Table I). Preoperatively, these

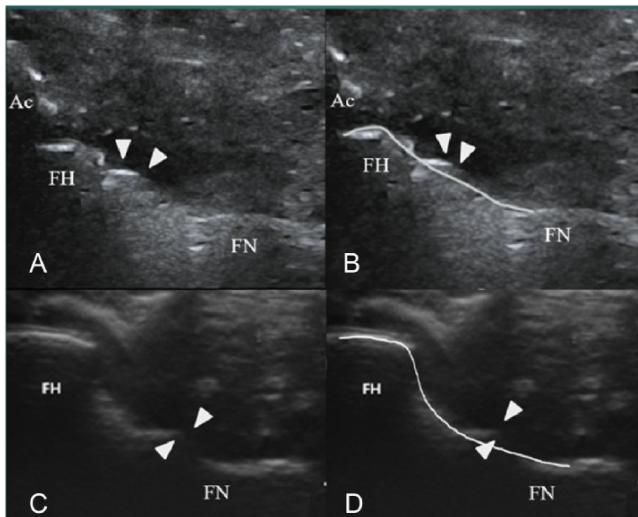


Fig. 4. Residual cam deformity under ultrasound. After the first osteochondroplasty, the residual cam deformity (arrowhead) presented as a distinctly irregular contour (A), and bony protuberance (C) was found under ultrasound. (B) and (D) White lines indicate the ideal FHNJ morphology, and the bone above the line indicates the residual cam deformity.

FN, femoral neck.

Table I. Patient information

	Hips, n (%) or mean \pm SD (range)	
	Ultrasound (n = 36)	Fluoroscopy (n = 39)
Sex		
Male	14 (38.9%)	11 (28.2%)
Female	22 (61.1%)	28 (71.8%)
Symptom duration before enrollment, months ($P = 0.593$)	33.5 (1,240)	19.4 (2,120)

SD, standard deviation.

Table II. Pre- and postoperative α angles measured via Dunn radiography

	Preopera- tive	Postopera- tive	P value
Ultrasound ($^{\circ}$)	59.4 ± 5.4	45.6 ± 4.0	$<0.001^{*}$
Fluoroscopy ($^{\circ}$)	59.4 ± 4.8	44.3 ± 3.3	$<0.001^{*}$
P value	0.703	0.128	

* $P < 0.001$.

two groups showed no significant differences in the α angle measured by Dunn radiography ($P > 0.05$; Table II). Preoperative CT images revealed no significant intergroup difference in the α angles measured at 1:00, 2:00 and 3:00 ($P > 0.05$). The maximal preoperative α angle occurred at the 2:00 position in the ultrasound and control groups ($59.3 \pm 9.4^{\circ}$ versus $60.3 \pm 9.5^{\circ}$, $P > 0.05$, Tables III and IV). In addition, cam deformity was most likely present at 2:00, with 27 (84.6%) patients in the ultrasound group and 33 (84.6%) in the control group showing cam deformity preoperatively.

Postoperatively, the Dunn radiographs showed significant reductions in the α angle in both groups, as the cam lesions had been removed ($P < 0.001$; Table III). However, no difference in the α angle measured in Dunn radiographs was found between the two groups ($46.4 \pm 4.6^{\circ}$ versus $44.3 \pm 3.8^{\circ}$, $P > 0.05$).

No difference in the residual cam deformity rate was observed between the two groups at 1:00, 2:00 and 3:00 ($P > 0.05$; Table IV). Residual cam deformity was most likely present at 1:00 in both the ultrasound (3/8.3%) and control groups (5/12.8%) (Tables III and IV).

Postoperative CT showed a tendency of decline in the α angle from 1:00 to 3:00 in both groups. No differences were found at the 1:00 and 2:00 positions between the two groups; however, the α angle of the ultrasound group at the 3:00 position was greater than that of the control group ($44.4 \pm 4.3^{\circ}$ versus $41.3 \pm 5.1^{\circ}$, $P < 0.05$; Tables III and IV).

The average operation time was 111.9 ± 20.5 min for the ultrasound group versus 105.2 ± 23.0 min for the control group ($P > 0.05$). None of the patients had complications, such as wounds or intra-articular infections.

DISCUSSION

Several studies have reported the use of ultrasonography in arthroscopy. Hua et al. first reported using ultrasound guidance to establish anterolateral and anterior approaches to hip arthroscopy with the patient in the supine position. In contrast, Keough et al. used intraoperative ultrasound to establish a hip arthroscopy approach with the patient in the lateral position [13, 19]. Our study focused on the possibility of using ultrasound to assess for residual cam deformity intraoperatively. Among the 39 cases, most FHNJs could be observed under ultrasonography during arthroscopy, suggesting the potential of ultrasonography as a tool to assess residual cam deformity intraoperatively. Unlike fluoroscopy, ultrasound can provide high-resolution images focusing on the morphological changes of the FHNJ and also different angles of observation. Residual cam deformities, such as bony protuberances, can be detected using intraoperative ultrasonography.

Osteochondroplasty performed during arthroscopy is a challenging procedure. Preoperative CT revealed that cam deformity was most likely present at the 2:00 position, causing the highest α angle at this position. However, postoperative CT showed a tendency of a decline in the α angle from 1:00 to 3:00 in both groups, leaving the 1:00 position as where the residual cam deformity was most likely to present. The surgeon was likely to remove more bone at 3:00 and less at 1:00, making the residual cam deformity most likely present at 1:00.

In this study, the α angle at the 3:00 position was greater in the ultrasound group than in the control group. Intraoperative ultrasound might have affected the surgeon's operative habits and reduced unnecessary bone removal at 3:00 because real-time and immediate observations with intraoperative ultrasonography provided surgeons with more opportunities to evaluate the bone while ensuring the removal of the deformity. Therefore, more normal bone was preserved during arthroscopy. Additionally, this normal bone tissue was not related to FAI, as our study found no evidence that ultrasonography increased or decreased the likelihood of residual cam deformity. We observed

Table III. Pre- and postoperative α angles measured on CT

	Preoperative			Postoperative		
	Ultrasound ($^{\circ}$)	Fluoroscopy ($^{\circ}$)	P	Ultrasound ($^{\circ}$)	Fluoroscopy ($^{\circ}$)	P
1:00	57.1 \pm 11.2	54.2 \pm 10.2	0.253	47.2 \pm 5.6	48.1 \pm 5.2	0.486
2:00	59.3 \pm 9.4	60.3 \pm 9.5	0.660	45.8 \pm 4.6	44.9 \pm 4.8	0.349
3:00	53.6 \pm 8.8	52.2 \pm 9.7	0.520	44.4 \pm 4.3	41.3 \pm 5.1	0.006*

*P < 0.05.

Table IV. Pre- and postoperative distribution of cam deformities

	Preoperative			Postoperative		
	Ultrasound	Fluoroscopy	P	Ultrasound	Fluoroscopy	P
1:00	16 (44.4%)	14 (35.9%)	0.450	3 (8.3%)	5 (12.8%)	0.799
2:00	27 (75.0%)	33 (84.6%)	0.413	1 (2.7%)	2 (5.1%)	>0.99
3:00	12 (33.3%)	16 (41.0%)	0.491	0 (0%)	0 (0%)	–

no significant difference in the α angle measured in Dunn radiographs between the two groups. Furthermore, there was no notable difference in the rate of residual cam deformity between the two groups at the 1:00, 2:00 and 3:00 positions.

The α angle was not measured using ultrasound intraoperatively to evaluate residual cam deformity. Buck et al. first developed a method to measure the angle with ultrasonography; however, their results did not support the use of ultrasound for angle measurement. Instead, an anterosuperior cam deformity is sensitive, and an anterosuperior bony protuberance is specific to cam FAI [5]. In addition, measurement of the α angle with ultrasound is time-consuming, and most ultrasound equipment does not have these built-in measurement tools. Therefore, we only observed and compared the morphologies of the FHNJs under ultrasound as in the control group. In our experience, this method is convenient, fast and practicable. None of the patients in the present study had complications, such as wounds or intra-articular infections. Furthermore, surgery time did not differ between the two groups, suggesting that the additional use of ultrasound is safe and does not cause severe surgical complications.

There are some limitations to the use of ultrasound. Ultrasonography may not obtain a clear image of the FHNJ during arthroscopy in all patients. Gas accumulation is a major problem intraoperatively, which may accumulate within the joint capsule and obscure the underlying structure. Filling the joint space with more saline could reduce the interference of air. Soft tissue swelling and surgical draping can also affect ultrasound imaging. The scan area should be preserved and exposed while draping. Intra-articular ultrasonography may provide a clearer image. Joukainen et al. described their experience in arthroscopic surgery using an intravascular probe to observe the hip structure intra-articularly [20]. Although the intravascular probe could be placed inside the articular hip, its image depth was not adequate for arthroscopic surgery, and these images required optimization. Because of the shelter of the osseous acetabulum, ultrasonography was employed to assess the cam deformity of the anterosuperior FHNJ. Considering that cam deformities predominantly

occur at the 1:00 (84.2%) and 2:00 (93.0%) positions in cam-type FAIs, ultrasonography proves sufficient for evaluating most patients [21].

Moreover, it is difficult for ultrasound to provide an objective measurement such as the α angle. A cadaveric study compared α angles measured on fluoroscopy with those on ultrasound at pre- and post-osteoplasty. The results showed a high level of agreement between the α angles on fluoroscopy and ultrasound at all positions in pre- and post-osteoplasty [22]. Daniel et al. described their methods of assessing cam deformity by measuring the α angle in multiple positions through intraoperative ultrasound [23]. However, based on our experience, intraoperative measurement of the α angle was challenging, especially when drawing the perfect circle around the femoral head, because only a small part of the femoral head is visible under ultrasound. Additionally, we would like to emphasize that the advantage of ultrasound is to provide continuous observation of the FHNJ and not angle measurements in several positions. Objective assessments like Hip Check from Stryker are not available under ultrasound. However, segmentation and assessment of the FHNJ based on artificial intelligence may be feasible under ultrasound in the future, as it is now being used to diagnose other diseases [24].

This study had some limitations. Intraoperative ultrasound was performed by a radiologist in this research, which means that a radiologist familiar with musculoskeletal ultrasound is required during surgery. However, the intraoperative technique used in this research was easy to learn. Once surgeons can identify the acetabulum and FHNJ under ultrasound, performing this technique themselves will not be difficult. Only one surgeon was involved in this research, and the risk of residual deformity was not high without ultrasonography. In addition, the number of patients included in this study was relatively small. Further studies, including more patients and surgeons of different seniority levels, are required to confirm the usefulness of intraoperative ultrasound. Only the α angle was measured to assess the morphologic outcomes of the surgery. However, we use a multiplanar reconstruction method and measure the α angle at 1:00, 2:00 and 3:00 to assess the global morphology of the FHNJ.

In conclusion, our findings suggest that ultrasound can help assess the condition of the FHNJ during arthroscopy. It does not reduce or increase the risk of residual cam deformity and positively affects the surgeon's performance by reducing unnecessary bone removal. Therefore, ultrasound is a safe and convenient tool for use during arthroscopy.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

None declared.

FUNDING

Beijing Natural Science Foundation (No. Z200003). The funder had no role in the design of the study or collection, analysis and interpretation of data or in writing the manuscript.

REFERENCES

- Hassan MM, Farooqi AS, Feroe AG *et al.* Open and arthroscopic management of femoroacetabular impingement: a review of current concepts. *J Hip Preserv Surg* 2022; **9**: 265–75.
- Kester BS, Capogna B, Mahure SA *et al.* Independent risk factors for revision surgery or conversion to total hip arthroplasty after hip arthroscopy: a review of a large statewide database from 2011 to 2012. *Arthroscopy* 2018; **34**: 464–70.
- Sardana V, Philippon MJ, de Sa D *et al.* Revision hip arthroscopy indications and outcomes: a systematic review. *Arthroscopy* 2015; **31**: 2047–55.
- Horner NS, Vikas K, MacDonald AE *et al.* Femoral neck fractures as a complication of hip arthroscopy: a systematic review. *J Hip Preserv Surg* 2017; **4**: 9–17.
- Aoki SK, Beckmann JT, Wylie JD. Arthroscopic femoral osteochondroplasty for cam-type femoroacetabular impingement: the trough technique. *Arthrosc Tech* 2016; **5**: e743–9.
- Buck FM, Hodler J, Zanetti M *et al.* Ultrasound for the evaluation of femoroacetabular impingement of the cam type. Diagnostic performance of qualitative criteria and alpha angle measurements. *Eur Radiol* 2011; **21**: 167–75.
- Lerch S, Kasprczyk A, Berndt T *et al.* Ultrasound is as reliable as plain radiographs in the diagnosis of cam-type femoroacetabular impingement. *Arch Orthop Trauma Surg* 2016; **136**: 1437–43.
- Lerch S, Kasprczyk A, Berndt T *et al.* Ultrasonography can quantify the extent of osteochondroplasty after treatment of Cam-type femoroacetabular impingement. *Int Orthop* 2015; **39**: 853–8.
- Troelsen A, Jacobsen S, Bolvig L *et al.* Ultrasound versus magnetic resonance arthrography in acetabular labral tear diagnostics: a prospective comparison in 20 dysplastic hips. *Acta Radiol* 2007; **48**: 1004–10.
- Lynch TS, Terry MA, Bedi A *et al.* Hip arthroscopic surgery: patient evaluation, current indications, and outcomes. *Am J Sports Med* 2013; **41**: 1174–89.
- Philippon MJ, Michalski MP, Campbell KJ *et al.* An anatomical study of the acetabulum with clinical applications to hip arthroscopy. *J Bone Joint Surg Am* 2014; **96**: 1673–82.
- Keough T, Wilson D, Wong I. Ultrasound-guided portal placement for hip arthroscopy. *Arthrosc Tech* 2016; **5**: e851–6.
- Albers CE, Wambeek N, Hanke MS *et al.* Imaging of femoroacetabular impingement-current concepts. *J Hip Preserv Surg* 2016; **3**: 245–61.
- Atkins PR, Shin Y, Agrawal P *et al.* Which two-dimensional radiographic measurements of cam femoroacetabular impingement best describe the three-dimensional shape of the proximal femur? *Clin Orthop Relat Res* 2019; **477**: 242–53.
- Leunig M, Podeszwa D, Beck M *et al.* Magnetic resonance arthrography of labral disorders in hips with dysplasia and impingement. *Clin Orthop Relat Res* 2004; **418**: 74–80.
- Kassarjian A, Yoon LS, Belzile E *et al.* Triad of MR arthrographic findings in patients with cam-type femoroacetabular impingement. *Radiology* 2005; **236**: 588–92.
- Mardones RM, Gonzalez C, Chen Q *et al.* Surgical treatment of femoroacetabular impingement: evaluation of the effect of the size of the resection. Surgical technique. *J Bone Joint Surg Am* 2006; **88**: 84–91.
- Nötzli HP, Wyss TF, Stoecklin CH *et al.* The contour of the femoral head-neck junction as a predictor for the risk of anterior impingement. *J Bone Joint Surg Br* 2002; **84**: 556–60.
- Hua Y, Yang Y, Chen S *et al.* Ultrasound-guided establishment of hip arthroscopy portals. *Arthroscopy* 2009; **25**: 1491–5.
- Joukainen A, Virén T, Penttilä P *et al.* Ultrasound arthroscopy of hip in treatment of osteochondritis Dissecans. *Arthrosc Tech* 2017; **6**: e1063–8.
- Khan O, Witt J. Evaluation of the magnitude and location of Cam deformity using three dimensional CT analysis. *Bone Joint J* 2014; **96-B**: 1167–71.
- Clapp IM, Alter TD, Sivasundaram L *et al.* Ultrasound demonstrates potential in identifying proximal femoral morphology before and after cam resection: a cadaveric study. *Arthroscopy* 2023; **39**: 751–7.e2.
- Kaplan DJ, Fenn TW, Larson JH *et al.* Intraoperative use of ultrasound for assessing cam deformity and cam resection. *Arthrosc Tech* 2023; **12**: e729–35.
- Xia Q, Cheng Y, Hu J *et al.* Differential diagnosis of breast cancer assisted by S-Detect artificial intelligence system. *Math Biosci Eng* 2021; **18**: 3680–9.