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ORIGINAL STUDY

Prediction of posttraumatic femoral head osteonecrosis by quantitative intraosseous aspirate and core biopsy analysis: A prospective study

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Twenty-two patients with unreduced hip dislocation or fracture dislocation were prospectively evaluated. Intraosseous aspiration, marrow fluid analysis and core biopsy histological analysis was performed from the supero-lateral (test group) and central part (control group) of the femoral head. After appropriate surgical treatment and postoperative management, they were followed up for 2 years by clinical, radiological and magnetic resonance imaging evaluation. Eight patients eventually developed avascular necrosis (AVN) of the femoral head. The analysis of test samples revealed that 9 patients had an aspirate volume of <1cc ; marrow morphology of 11 hips showed necrotic cells ; 12 patients had a core biopsy histology suggestive of dead osseous fragments and necrotic osteocytes. In contrast, all the control samples had an aspirate volume of >1cc and showed viable cells on histology. Intra-operative assessment of marrow-aspirate volume (<1cc), marrow morphology and core biopsy histology from the superolateral part of femur head can fairly predict the development of subsequent ONFH after trauma ; the correlation is statistically significant (p<0.05).

Key words: avascular necrosis ; posttraumatic osteonecrosis ; femur head ; aspiration volume ; core biopsy ; marrow morphology.

INTRODUCTION

Various aetiological factors, traumatic and non-traumatic, are responsible for the development of avascular necrosis (AVN) of the femoral head. Displaced fractures of the neck of the femur and hip dislocations are the usual traumatic events leading to such complication (*1,10*). Early reduction is the most effective means of preventing osteonecrosis in hip dislocations. Hougaard and Thomsen reported a 4.8% incidence of osteonecrosis in hips reduced within 6 hours and 52.9% when reduction was delayed for more than 6 hours after injury (*4*).

Avascular necrosis, if not diagnosed in its early stages, progresses in severity and ultimately leads to osteoarthritis. This is the most frequent reason for undergoing arthroplasty in young individuals in

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the United States. An early diagnosis of AVN in this context is essential, as a joint preserving procedure (i.e core decompression, muscle pedicle graft, vascularised fibular graft etc.) can be performed (*1,10*). In contrast to non-traumatic aetiology, the onset of avascularity is defined in traumatic osteonecrosis, so timely investigations can diagnose the existence of the earliest possible alteration in the vascularity of the femoral head. The general concept is that cellular changes precede structural changes in vascular insults and the supero-lateral area of the femur head is the most susceptible site for ischaemic necrosis (*1,13*). The common investigative tools like radiographs, bone scan and MRI can detect the structural changes of the femoral head in AVN after a few days or weeks following injury, but the delay in diagnosis with these investigation modalities may not permit a hip salvage procedure in all cases (*1,2,5,10,12*).

As the supero-lateral area of the femur head is the usual site of osteonecrosis, we hypothesized that this area might have less perfusion and in an ischaemic condition it would yield less aspirate volume. Intra-operative assessment (patients who require surgical intervention for posterior dislocation of the hip) of this aspirate volume, would predict the subsequent development ON, thus making it possible to consider a joint preserving procedure at an early stage. The presentation of many cases of neglected dislocation of hips requiring open reduction provided us an opportunity to assess this hypothesis.

MATERIALS AND METHODS

Study design and patients selection criteria

In a one-year prospective study conducted at our institution, all patients attending the orthopaedic trauma service with an unreduced dislocation or fracture dislocation of the hip more than 12 hours after injury and requiring surgery for reduction of the joint dislocation, were included. The patients were within the age group of 20-50 years and without any history of prolonged steroid use, chronic alcoholism or diseases such as systemic lupus erythematosus, sickle cell disease, Gaucher disease or any malignancy. A total of 22 patients were recruited. Institutional Medical ethics permission and consent of the patients were obtained before their enrollment in this study.

A predesigned proforma was prepared in which the general profile of the patients, mode of injury, any associated co-morbidities, local area examination and radiological findings were documented. As part of the injury care, these patients were operated upon for the relocation of the femoral head as well as for the repair of the associated fractures if any. Intraoperatively, the superolateral part of the femoral head was delineated with the help of a C-arm. Then two samples each of aspirates and core biopsy were obtained from the superolateral part (test) and middle part (control) of the femoral head for histological evaluation.

Test for the vascular status of the femoral head

Aspiration Test

A bone marrow aspiration needle (bore size 20G) with the stylet *in situ* was inserted from the non articular area of the femoral neck into the femur head with adequate insertion depth to reach its supero-lateral weight bearing area under C-arm image control. The stylet was then removed and marrow was aspirated with a 50 cc syringe with negative pressure (full plunger pull) maintained for a period of 60 seconds. The aspirate volume was measured and put in an EDTA vial for cytological analysis and differential cell count. Two more aspiration samples were further drawn from two sites around the middle part of the femoral head; the aspirate was measured and sent for cytological analysis.

Needle bone biopsy

The bone biopsy needle (size 2 mm diameter) was used to obtain a biopsy from the supero-lateral part and then a control sample from the center of the femoral head. The bone samples thus obtained from the weight bearing area of the femoral head were kept in 10% formalin for subsequent histological evaluation. The bone tissue was decalcified and stained with H & E stain and microscopically examined for cellular and stroma characteristics.

After appropriate surgical treatment (open reduction and whenever required internal fixation using titanium implants through a posterior approach by one senior surgeon), all patients were managed as per standard protocol for dislocation and associated fracture dislocation of the hip joint.

Follow-up Evaluation

These patients were followed up at months 1.5, 3, 6, 12 and every year thereafter. The average follow-up period was 2 years (range : 1.5 to 3.2 years). At each follow-up, patients were subjected to complete clinical, radiological and MRI evaluation of the affected hip. MRI of the hip was conducted for diagnosis of AVN as per Mitchell's criteria (7) : an area of low signal or a dark line on T1 weighted image and a double line on T2 weighted image (inner bright line & outer dark line).

All patients showing MRI changes suggestive of AVN in two consecutive scans were labeled as having AVN of the femoral head. An attempt was then made to correlate histology and volume of aspirate of the femoral head with subsequent AVN changes.

Statistical analysis

Pearson Chi Square test was performed to analyze the relationship between the injury factors like delayed reduction and type of reduction whether open or closed, with investigations of aspirate volume and morphology. Regression analysis was performed to know whether the investigational interventions like aspiration volume, marrow morphology and core biopsy histology were predictive of the outcome. The correlation was taken as significant if p value was found to be less than 0.05.

RESULTS

The mean age of the patients was 39.4 ± 9.4 years (range : 20 to 50 years). Twenty patients (90.9%) were male. Along with posterior dislocation of the hip, 19 patients had an associated fracture of the posterior wall of the acetabulum, two had an associated femur head fracture and one had combined acetabular posterior wall and femoral head fracture. Twelve patients (54.5%) with hip dislocation were reduced within 12 to 24 hours, 3 (13.6%) within 24 to 72 hours and 7 (31.8%) were reduced after 72 hours from injury. In 14 of the 22 patients, (63.6%) closed reduction had been achieved before surgery for the stabilization of the fracture. The remaining 8 patients (36.4%) needed open reduction.

Test samples

Aspiration volume : The aspirate of less than 1 cc from the supero-lateral aspect of the femoral head was labeled as ischaemic head. Nine patients (40.9%) had aspirate volume less than 1 cc whereas 13 patients (59.1%) had aspirate volume more than 1 cc.

Marrow analysis : The cellular morphology was taken as an indicator of ischaemia if it showed the presence of necrosis of haematopoietic marrow and adipocytes. Similar ischaemic cell characteristics were noted in 11 cases (50%) whereas viable cells were found in the remaining 11 cases (50%).

Histological analysis : The presence of liquefaction necrosis and interstitial oedema, with osteocyte necrosis in biopsy was defined as ischaemia. We found 12 patients (54.5%) showing osteocyte necrosis on histology of the core biopsy ; the other 10 patients (45.5%) showed viable cellular characteristics.

Control samples : All the aspirates from the central part of the head had a volume of > 1cc. The marrow and core biopsy histological analysis revealed viable cells in all control samples.

By the end of two years, 8 patients had already developed MRI evidence of AVN. Staging was done using the criteria of the Association Research Circulation Osseous (ARCO) Committee. Seven patients were in stage I and one had stage III AVN. Six patients (in stage I) were diagnosed within 1.5 month, one within 3 months (stage III) and the remaining one (stage I) showed scan evidence of AVN after a longer time interval. One patient underwent total hip replacement (THR) and the others were treated by core decompression. The core biopsysamples were taken for histological proof of AVN.

The relationship between less aspirate volume, marrow and core histological characteristics for ischaemia and necrosis was analyzed (tables I, II, III). The correlation matrix showed significant correlation of histology with aspiration volume and marrow characteristics (correlation matrix 0.574 and 0.436). Sex, duration of dislocation and type of reduction (closed or open) did not show any significant correlation with eventual development of AVN. However all these three investigation modalities (aspiration volume, marrow characteristics and histology) showed significant correlation ($p < 0.05$) with subsequent development of AVN.

Prediction of avascular necrosis by Logistic Regression analysis

Logistic regression analysis was used for the prediction of avascularity of the femoral head, among the patients in relation with the duration of dislocation, the reduction method whether closed or open, the volume of aspirate, marrow characteristics and histological analysis (tables I, II, III). The association was observed to be non-significant for duration of dislocation and reduction type. However prediction of AVN was high using aspirate volume and marrow quality ($p < 0.05$) as the investigative tool. As a predictor of AVN, the sensitivity of aspirate volume was 100% and the specificity was 92.9%.

DISCUSSION

The diagnosis of post traumatic AVN of the femoral head is usually made once the patient becomes symptomatic after a few months of recovery from the initial event. At this stage radiographic changes can be seen in the femoral head as lucency or subchondral sclerosis suggesting AVN. Once this stage of AVN appears in the femoral head, the treatment modalities do not permit salvage of natu-

Table I. — Analysis of observations

A. Correlation of duration of dislocation with aspiration volume, marrow characteristics, core biopsy histology and AVN by MRI evaluation

	Aspirate volume		Total	Marrow morphology		Total	Core biopsy histology		Total	AVN (ARCO)			Total
	< 1cc	> 1cc		ischaemic	viable		necrosis	normal		normal	1	3	
Duration 1 (12-24hrs)	6	6	12	6	6	12	5	7	12	7	5	0	12
2 (24-72 hrs)	0	3	3	1	2	3	1	2	3	2	1	0	3
3 (>72 hrs)	3	4	7	4	3	7	6	1	7	5	1	1	7
Total	9	13	22	11	11	22	12	10	22	14	7	1	22
Pearson Chi-Square	2.498 (a)	2	0.287	0.476 (a)	2	0.788	4.090 (a)	2	0.129	3.311(a)		4	0.507

a : cut off value 0.500

B. Correlation of method of reduction with aspiration volume, marrow characteristics, core biopsy histology and ON by MRI evaluation

	Aspirate volume		Total	Marrow morphology		Total	Core biopsy histology		Total	AVN (ARCO)			Total
	< 1cc	> 1cc		ischaemic	viable		necrosis	normal		normal	1	3	
Reduction Closed	4	4	8	5	3	8	6	2	8	9	5	0	14
Open	5	9	14	6	8	14	6	8	14	5	2	1	8
Total	9	13	22	11	11	22	12	10	22	14	7	1	22
Pearson Chi-Square	0.430	1	0.512	0.786	1	0.375	2.121	1	0.145	1.936	2		0.380

Table II. — Correlations between quantitative aspirate volume, marrow morphology and histological characteristics

		Aspirate Volume < 1 cc	Marrow morphology of necrosis	Histology of ischaemia
Aspiration volume	Pearson Correlation	1	0.203	0.574(**)
	Sig. (2-tailed)		0.366	.005
	N	22	22	22
Marrow analysis	Pearson Correlation	0.203	1	0.436(*)
	Sig. (2-tailed)	0.366	0.043	
	N	22	22	22
Histology	Pearson Correlation	0.574(**)	0.436(*)	1
	Sig. (2-tailed)	0.005	0.043	.
	N	22	22	22

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

ral hip, and reconstruction procedures are usually required (*1,10*).

An early diagnosis of AVN is important to minimize complications of the advanced lesion, and to preserve the option of conservative treatment modalities with better outcome (*1,10*). As described earlier, after vascular damage, osteonecrosis starts with bone cell ischaemia followed by structural changes. Thus prediction of AVN can be established at the earliest period, by the assessment of aspiration volume and cytological analysis in the ischaemic area. The present study showed that aspirate volume of < 1cc, marrow characteristics and histology are investigative tests that can potentially predict subsequent AVN changes in the femoral head ($p < 0.05$). However the histological analysis of marrow samples and core biopsy specimens require processing, appropriate laboratory support and expertise. These two modalities cannot be a part of routine intra-operative injury care. In comparison, the simple estimation of aspirate volume is a simple tool to diagnose femoral head ischaemia. Patients with hip dislocation/fracture dislocation are at risk to develop AVN. This vulnerable group can be further categorized into two group based on marrow aspirate volume (< 1 cc or > 1 cc). Patients with less than 1cc marrow aspirate are at a relatively higher risk to develop osteonecrosis and should undergo early imaging of the hip so that a hip salvage procedure can be performed. Seven of the eight osteonecrotic hips in the present study were treated with core decompression as a close observation with regular frequent follow-up could detect the lesion at ARCO stage 1 of AVN. Because of the short follow-up period, the present study could not evaluate the long term effectiveness of core decompression in these patients. However they are still under our follow-up.

Aspiration of the femoral head for vascularity assessment in fracture of the neck of the femur, as proposed by Gill *et al* had 100% predictivity (*3*). In agreement with the literature, this study also supports the aspiration method as a valuable diagnostic tool with high level of accuracy in posterior dislocation of the hip. Yue *et al* reported that ORIF of acetabular fractures using a Kocher-Langenbeck or ilioinguinal operative approach does not seem to affect blood flow to the femoral head in the immediate postoperative period (*15*). The surgical approach in all of our patients remained standard and uniform (posterior approach).

Numerous intra-operative methods have been described for prediction of avascularity of the femoral head. Watanabe *et al* measured the intramedullary oxygen tension of the femoral head in patients with acute femoral neck fracture while treating them with internal fixation. Avascular necrosis was diagnosed by magnetic resonance

Table III. — Correlation of several variables with eventual development of AVN (A)

		Score	Df	Sig.
Variables				
	Duration	0.014	1	0.907
	Reduction	0.007	1	0.933
	Aspirate	6.044	1	0.014
	Marrow	12.571	1	0.000
	Histology	10.476	1	0.001
Overall Statistics		16.748	6	0.010

Prediction of AVN by aspirate volume (B)

Observed		Predicted		
				Percentage Correct
		normal	ARCO grade	
ON	absent	13	1	92.9
	present	0	8	100.0
Overall Percentage				95.5

imaging (MRI) performed at 2, 6, and 12 months after the surgery. The authors observed that there was significantly low oxygen tension at a point near the joint surface compared to a point proximal to the fracture site and so concluded that the method of measuring intramedullary oxygen tension has the possibility for intra-operatively identifying a risk group for development of AVN (*13*). Matsumoto *et al* have used an electrochemically generated hydrogen clearance method to assess the femoral head vascularity ; they concluded that it could indeed predict the alterations in its blood supply (*6*). Notzli *et al* (*9*) and Sugamoto e*t al* (*11*) measured the perfusion of the femoral head by Doppler flowmetry with a high-energy laser intraoperatively and observed changes in vascularity with various femur head positions. Similarly Nakamura *et al* (*8*) proposed the use of PET scan for evaluation of the haemodynamics of the femoral head. Polarographic determination of O2 pressure in the femoral head is another investigative tool for prediction of AVN (*14*).

In all these studies however, specific sets of investigation tools are required and these are not cost-effective. Accordingly this may not be possible for routine use in most hospital situations. In comparison to these, the assessment of aspiration volume from the superior part of the femoral head is an easy, clinically applicable, cost effective procedure for assessment of the ischaemic status of the femoral head.

Limitations of the present study include the small sample size and short follow-up. As a pilot study, the present report highlights some important observations in relation to early diagnosis of osteonecrosis by marrow aspirate and biopsy and stimulates researchers to undergo more elaborate studies. Another limitation is the absence of a definition of what normal aspirate is and what exactly should be the cutoff level for determination of an ischaemic status. In the present study the choice of 1 cc was decided upon preliminary observations of minimal aspirate (less than 0.5 cc) in many of these cases with delayed reduction of hip joints ; the observations in the present study have supported this perception.

In conclusion, we believe that intra-operative measurement of aspirate volume from the superolateral part of the femoral head can fairly predict subsequent avascular necrosis in patients sustaining a posterior dislocation or fracture dislocation of the hip. This method is simple and easy to perform, clinically applicable and cost effective. Less than 1 cc of marrow aspiration from the superior part of the head, using a 50 cc syringe with negative pressure maintained for a full minute can be taken as an indicator of ischaemia. This relatively high risk group needs frequent follow-up and early imaging, so that a hip conserving procedure can be considered at an early stage.

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