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Research paper

Study of neuroreceptors in native ACL stump and autologus hamstring tendon graft

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ABSTRACT

Background: Anterior cruciate ligament (ACL) reconstruction is a very common arthroscopic surgery. All around the world the commonest autograft preferred is hamstring tendon. Present trend is more in favor of retaining the native ACL stump as the available neuroreceptors in the stump might help in early recovery of proprioception after ACL reconstruction.

Methods: This is a prospective study done on 56 cases. We took biopsy samples from knee joints of patients who had undergone arthroscopic ACL reconstruction at our hospital from native ACL stump as well as the graft (Semitendinosus) and sent them for immunohistological examination using S-100 Protein and NFP (Neural filament protein).

Results: Chronicity of ACL deficient knee's (injury to surgery more than 6 months) revealed poor positivity for neuroreceptors in sample A. After 3 months of injury there was a gradual decrease in positivity for neuroreceptors with persistence of these neural elements upto 6 months within remnant ACL stump.

Conclusion: The results suggest that complete or partial ACL tears should be addressed by early arthroscopic ACL reconstruction using remnant-preserving technique, as the number of mechanoreceptors gradually deteriorates with time following injury.

1. Introduction

Palmer and Abbott described ACL as a double bundle structure, the anteromedial (AM) and the posterolateral (PL) bundle, which provide anterior and rotational stability of the knee.[1,2] The tibial footprint dictates the nomenclature of the two ACL bundles.[3,4] ACL reconstruction was first described by Hey Groves in 1917.[5] Single bundle (SB) ACL reconstruction has given good outcomes in restoration of anteroposterior knee stability in most patients.[6,7] Tashman et al. evaluated patients who had undergone single bundle ACL reconstruction and concluded that SB restored anteroposterior knee stability, but not rotational stability, of the knee joint.[8] Double bundle (DB) ACL reconstruction provides both anteroposterior knee stability and rotational stability. However, in literature there is no consensus on which technique is superior.[10]

Remnant preserving ACL reconstruction has been and topic of discussion and controversy. Several authors recommend shaving of ruptured stump of ACL for better accommodation and visualization of a good-sized graft, especially a DB graft.[9,11,12] Whereas others feel that in remnant preserving technique the available neuroreceptors help in early recovery of proprioception.[13–17] There are three reasons to preserve the remnant stump: biomechanical advantage, better vascular and proprioceptive recovery.[13–17] Remnant ACL stump provides ingrowth of mechanoreceptors resulting in early proprioceptive recovery following ACL reconstruction.[13–17] Chronicity of ACL tear affects the availability of neuroreceptors.[18] Denti et al. demonstrated that the mechanoreceptors within ACL gradually decrease in number after 3 months of injury and could not be demonstrated after one year of injury.[18] Whereas, Dhillon et al. showed persistence of mechanoreceptors in one of the cases as late as 42 months after rupture.[19] Crain et al. classified ACL remnant stumps in to four types: Type 1 native ruptured ACL remnant adherent to PCL, Type 2 remnant tissue healed to roof of the notch, Type 3 healed to lateral wall and Type 4 complete resorption of stump ACL.[20]

1.1. The purpose of present study

The purpose of this study was to find out the presence of neuroreceptors within ruptured native ACL stump and autologous hamstring graft (semitendinosis) (Fig. 1). This study used S-100

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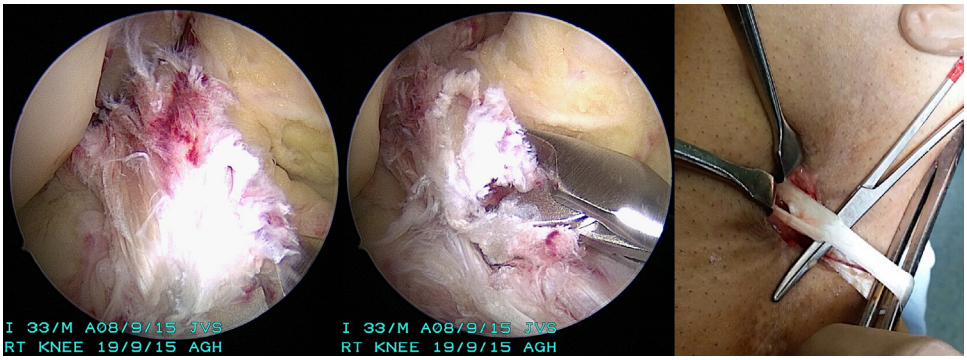

Fig. 1. (A) Arthroscopic view of native ruptured ACL stump. (B) Biopsy taken using a 1.5 mm basket punch from native ACL stump (Sample A) close to its tibial attachment site. (C) During graft preparation biopsy Sample B (semitendinosus tendon) was taken close to its insertion.

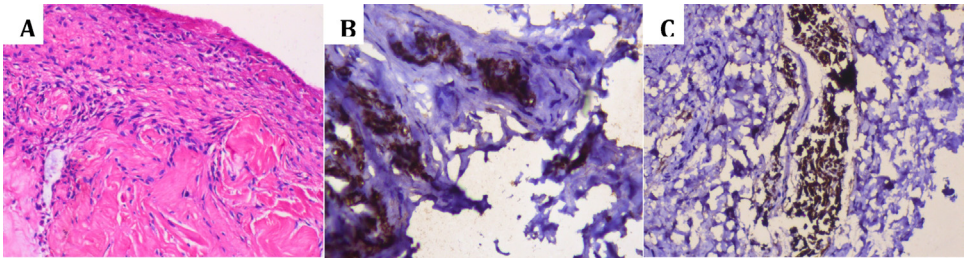

Fig. 2. (A) Haematoxylin and eosin (H&E) stained section showing structure of native ruptured ACL stump H&E 200. (B) Grade III Neuroreceptors IHC with S-100 protein X 200. (C) Grade I Neuroreceptors IHC with Neurofilament protein (NFP) X 100.

Protein and NFP, immunohistological markers, to identify neural elements (Fig. 2).

2. Material and methods

The study is a prospective study comprising of 56 consecutive patients who presented to us with complete ACL tear for whom arthroscopic ACL reconstruction was done from January 2012 to July 2015.The study was approved by the ethics committee of our institution and all patients provided written informed consent to participate in this study.

Inclusion criteria: clinically unstable, ACL deficient knee, confirmed on MRI, with pivot shift positivity.

Exclusion criteria: Multiligamentous injuries, previously operated knees, severe Osteoarthritis, non-availability of ACL stump for biopsy.

Out of the 56 patients, 38 were male and the rest 18 were female. 34 patients had right knee injury and 22 patients had left knee injury. There were 26 patients within the age group 20–35 years, 24 patients in the age group 35–50 years and six patients belonged to more than 50 years of age. Patients were grouped based on the time of injury and the time ACL reconstruction was done within 6 months interval, 6 months to 1 year and more than 1 year time period.

The surgical technique was performed under spinal anesthesia; diagnostic arthroscopy was done using lateral and medial arthroscopic portals with a 30^0 fore oblique arthroscope. At arthroscopy,biopsy was taken from native ruptured ACL stump (Sample A) close to its tibial attachment site using a 1.5 mm basket punch (Fig. 1). Cases with chronic ACL rupture with no available stump, biopsy was not taken. They were excluded from the study. During graft preparation Sample B was taken from semitendinosus tendon (ST) close to its tibial attachment site (Fig. 1).

The samples were preserved in 10% formaldehyde solution. These Samples were processed routinely and paraffin embedded. Sections were stained using haematoxylin and eosin (H&E), immunohistochemistry was done using S-100 protein and Neurofilament protein (NFP) antibodies (Fig. 2). The reason for using these IHC markers was their potency in identifying neuroreceptors. S-100 protein is located in Schwann cells and myelinated fibers whereas NFP is located in axon cytoskeleton.[20]

3. Results

A total of 56 cases of ACL reconstruction were taken up for the study. All specimens were stained by H&E and IHC markers. The patients were grouped based on the time of injury and when the ACL reconstruction was done. There were 45 patients within the time interval 6 months, six patients were in 6 months to 1 year and five patients were more than 1 year time period. The patients within the 6 months time interval of injury showed presence of neuroreceptors, whereas patients after 6 months did not show any neuroreceptors.

The area of the field was 0.65 mm and a subjective grading of number of positive areas/HPF was counted and reported. Presences of neuroreceptors were classified as follows: group I (+1/HPF), group II (+2/HPF) and group III (+3/HPF) (Table 2). Patients more than 6 months duration, showed negative grading for the presence of neuroreceptors. Whereas, patients who were within the 6

Table 1
Results of Immunohistological analysis of Sample A (native ACL stump) and Sample B (Semitendinous tendon graft) using S-100 protein and Neurofilament protein (NFP).

S-100 Protein	Positive for Neuroreceptors	Negative for Neuroreceptors
SAMPLE – A	38(67.86%)	18(32.14%)
SAMPLE – B	41(73.21%)	15(26.74%)
NFP		
SAMPLE – A	41(73.21%)	15(26.74%)
SAMPLE – B	44(78.57%)	12 (21.43%)