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Relevance of Intra - Operative Vancomycin Hamstring Autograft Soaking in Primary Anterior Cruciate Ligament Reconstruction

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Abstract: ***Background: To investigate the relevance of hamstring autograft soaking with vancomycin reduces postoperative infection rate after primary Anterior Cruciate Ligament Reconstruction (ACLR). Objective(s): To compare the rate of postoperative infection rate between the vancomycin graft soaking group and non-soaking group, as well as to find the association between patient demographic factors (age, gender, BMI, smoking, and duration of surgery) with postoperative infection following primary ACLR. Method: Retrospective medical record review of all primary ACL reconstructions using hamstring autograft over a 4-year in a government hospital. The patients only received standard prophylactic IV antibiotics in the initial 2-year period (Group A). In the following 2-year period, the patients received prophylactic IV antibiotics and a vancomycin solution soaked into the graft (Group B). Results: There were 74 patients, of which 33 were in Group A, and 41 were in Group B, respectively. Five cases of postoperative infections were identified in the population (6.76%). Four patients were found in group A, contributing a rate of infection of 12.12% in comparison with 1 case of postoperative infection (2.44%) in group B (p > 0.005). In the model to estimate the association of patient demographic factors (age, gender, BMI, smoking, and duration of surgery) between postoperative infection rate in autograft soaking with vancomycin group and non-soaking autograft group in primary ACL reconstructions, we found there were no statistically significant risk factors in our analysis (p-value >0.25). Conclusions: The additional technique of hamstring autograft soaking with vancomycin solution compared to a single prophylactic IV antibiotic could reduce the incidence of postoperative infection after primary ACLR. Antibiotic graft soaking may be relevant as an additional prophylaxis measure to prevent postoperative infection after ACLR.***

Keywords: Vancomycin, postoperative infection, anterior cruciate ligament, graft soaking

1. Introduction

Anterior cruciate ligament reconstruction (ACLR) is a more commonly performed orthopaedic procedure. However, postoperative infections can be a severe complication. Judd et al. have reported the rate of infections after ACLR in 0.14% to 5.7% of surgeries [6]. Maletis et al. determined that the overall Surgical Site Infection (SSI) rate after ACLR was 0.48%, of which 0.32% involved deep infections and 0.16% were superficial infections [4]. Most reported postoperative deep infection cases are caused by strains of the *Staphylococcus* genus, particularly by *Coagulase-negative Staphylococcus aureus* (CONS), such as *Staphylococcus epidermidis* [6,7]. Meanwhile, septic arthritis following primary Anterior Cruciate Ligament Reconstruction remains one of the most devastating complications despite improvements in the operative environment and surgical techniques. The incidence rate ranges from 0.14 to 0.78 % [1, 2].

The etiology of SSI is multifactorial. For an infection to occur, bacterial contamination or colonization and an environment conducive to bacterial growth must occur. Several studies investigated the colonization of microorganisms and operative contamination, mainly dealing with patients undergoing joint arthroplasties. Nakayama et al. reported the presence of microorganism skin colonization in 46% of patients undergoing ACL reconstruction during the preoperative examination, whereas 6% of the skin swabs and 2% of the swabs taken from the graft showed positive results intraoperatively [5]. Bacterial contamination during surgery is also an essential factor, and it may come from exogenous sources. The contaminating skin commensals such as *Coagulase-negative Staphylococcus aureus* (CONS) and *Streptococcus species* are transferred mainly through the operation theatre staff to various sites in the operative field. Plante et al. reviewed hamstring tendon autografts and found positive cultures in 23% of harvested grafts [10]. In addition, the contamination of tendon autografts can occur during the harvest process or even when the passage of grafts into the tunnels through the arthroscopic portals [1]. Parada et al. identified contamination in the cannulated portion of the screwdriver used to insert the tibial screw and sheath as the source of an outbreak of infections with hamstring tendon grafts [8]. Furthermore, Byrne et al. found that the contamination rate was 17.8% from skin swabs, 3.0% from skin blades, 1.3% from the inside blade, 4.6% from the suction tip, and 4.4% from the suture line [9]. The contamination is also coming from glove tips, syringes, gowns, and light handles, which could be potential risks for postoperative infection [11].

Many centers worldwide practiced the intra-operative autologous hamstring graft soaking with vancomycin antibiotics in primary ACLR. The practice is an additional precaution, along with using a preoperative intravenous prophylactic antibiotic, which has efficiently reduced the postoperative infection rate [13]. The hamstring autograft presoaking with vancomycin has shown promising results in decreasing the infection rate following an ACLR [15].

Therefore, this study is performed to enhance the understanding of reliability regarding autograft soaking with vancomycin in primary ACLR. The main purpose of this study was to find out and investigate the effect of prophylactic graft saturation with vancomycin in reducing the infection rate following an ACLR using hamstrings autografts, which may suggest a standard protocol in our institution. We want to determine the incidence of postoperative infection in primary ACLR in our hospital and to assess the association between postoperative infection rate in autograft soaking with vancomycin group and non-soaking autograft group in primary ACLR. In the presence of the patient's risk factors, we also want to know their association with the incidence of postoperative infection. The initial hypothesis is that prophylactic autograft presoaking in vancomycin combined with a prophylactic intravenous (IV) antibiotic reduces the incidence of postoperative infection after ACLR.

2. Materials and Methods

A retrospective medical record review of all the patients that consecutively underwent primary arthroscopic ACLR with a hamstring autograft in Hospital Sultan Ismail from 1 January 2017 to 31 December 2020 was performed. This study was entirely using retrospective data extracted from medical records. Hence, there was no direct subject participation. This study required no sampling as all patients in the inclusion criteria were enrolled. The study did not include patients who needed an extra-articular procedure or those who received bone-patellar-tendon-bone autograft or an allograft. The revision cases or patients with a history of septic joint infection or knee joint arthrotomy washout were also excluded. In the initial 2-year period, the patients received preoperative IV antibiotics (Group A). In the following 2-year period, the patients received preoperative IV antibiotics and a vancomycin solution presoaked into the graft (Group B). All detailed information, including the patient's demographic and surgery, was recorded in the designated study form. The authors traced the postoperative culture results from septic arthritis (if any) via Microbiology Lab within the same registry system.

All patients were operated on by the Orthopedics Sport Unit led by a senior surgeon. The surgeon performed all operations with similar surgical techniques on an inpatient basis. The prophylactic antibiotic protocol consisted of a single dose of 1.5 g of preoperative IV cefuroxime. The assistant poured a solution of 100 ml of sterile normal saline into a tray and saturated with 500 mg of vancomycin powder. Meanwhile, the graft was harvested and prepared in quadrupled hamstring. The vancomycin solution soaked the prepared graft in the tray, then wrapped in a wet gauze that was saturated with the solution beforehand. The hamstring graft was left there for 15 minutes, followed by a pre-tensioning of 80 N for 5 minutes before the ACL fixation. This vancomycin saturation technique is performed as Grayson et al. described [15]. The surgeon used a cortical suspensory fixation system (XO Button Fixation System) for the femoral fixation of the graft, whereby he used a resorbable interference screw for the tibial fixation of the graft. The surgeon did not practice the intraarticular drain insertion. Postoperatively, the patient was given antibiotics IV cefuroxime 750mg TDS prophylaxis for three days. The surgical team inspected the incision wounds on Day 3 post-operation before being immediately cleaned and sealed with a waterproof transparent dressing. Standard wound care is performed every other day for two weeks at outpatient health clinics.

Diagnosis of SSI was based on the patient's symptoms and physical examination, laboratory parameters, and cultures of synovial fluid or joint tissue, according to definitions stated in the guidelines issued by the National Nosocomial Infections Surveillance System [14]. A superficial SSI diagnosed within four weeks after the surgery was considered a postoperative infection. A deep SSI was attributed to the procedure if it occurred up to 1 year postoperatively, involving deep soft tissue. In the case of septic arthritis, the surgeon performed the synovial aspiration as soon as the diagnosis was suspected. The synovial fluid obtained was immediately sent for biochemical analysis and cell count analysis in sealed containers. The laboratory alsoperformed an antibiotic sensitivity test on the specimen.

We performed the data analysis using the SPSS version 22 using descriptive statistics. The parametric numerical data are expressed as mean ± standard deviation (SD). The non-parametric data are expressed as a median and interquartile range (IQR). The simple/ multiple logistic regression tests are used to investigate the association (Odds Ratio) between independent factors (demographic, BMI, smoking status, and duration of surgery) and the surgical outcome (postoperative infection) as dependent variables. The Pearson Chi-square or Fisher exact test is used to look for the association between dependent factors (demographic, BMI, smoking status, and duration of surgery) and the surgical outcome (postoperative infection versus no infection), and a p-value of <0.05 is considered statistically significant.

3. Results

There were 74 patients with 26 mean ages and 8.34 standard deviation (Table 1). The male was the most significant proportion in this study, with 66 patients (89.2%) and balanced only eight patients (10.8%). Nineteen patients (25.7%) were smokers, and nine patients (12.2%) had a BMI of more than 30 kg/m² before ACL reconstruction. There were 21 cases of isolated ACL tears (28.4%), whereas 53 cases (71.6%) were ACL tears associated with meniscus tears. The mean duration of ACL reconstruction was 132.14 minutes and 29.32 standard deviations. This study's overall infection rate was 6.8% (5 cases).All cases were acute infections which were reported within three weeks postoperative period. We found four infection cases in Group A (graft non-soaking), which represents a 12.12% rate of infection (4 out of 33). One infection case occurred in Group B (graft presoaking with vancomycin). It meant a rate of infection of 2.44% (1 out of 41). Two infection cases were deep SSI, one from Group A and B. The surgeon successfully treated both cases with an arthroscopic arthrotomy washout. The remaining infection cases were superficial SSI which were resolved with antibiotics treatment. We found no significant differences between the

risk factors in the descriptive statistics of the patients with postoperative infection rates in both Group A and Group B (Table 3). The highest proportion of patients with autograft soaking was male, with 37 patients (90.2%) with BMI less than 30kg/m²(90.2%). In our analysis using a simple logistic regression model to estimate the associated factors related to postoperative infection rate in between autograft soaking with vancomycin group and non-soaking autograft group (Table 2), we found that the postoperative infection possibly had an association between these two groups, where p-value <0.25. However, we could not find any statistically significant association of risk factors of smoking, high BMI, and prolonged duration of surgery in the analysis (p-value >0.25). Next, we included the variables with p-value <0.25 and biologically or clinically crucial from simple logistic regression in multiple logistic regression. As a result, we found that all variables, including the postoperative infection, showed no statistically significant differences in the primary effect model (p>0.05). Therefore, in our study, there was no statistical difference that soaking the ACL graft in vancomycin significantly reduced the postoperative infection rate (p > 0.05) compared to patients who received only IV prophylactic antibiotics.

Table 1: The sociodemographic characteristics of patients who underwent primary arthroscopic ACLR with hamstring autograft in Sultan Ismail Hospital during the period 2017 to 2020 (n=74)

Variable	n(%)
Age	26.86 (8.34)
Gender	
Male	66 (89.2)
Female	8 (10.8)
BMI(kg/m²)	
<30	65 (87.8)
>30	9 (12.2)
Smoking status	
No	55 (74.3)
Yes	19 (25.7)
Diagnosis	
ACL tear only	21 (28.4)
ACL tear withmeniscus tear	53 (71.6)
Duration of surgery	132.14 (29.32)
Postoperative Infection	
No	69 (93.2)
Yes	5 (6.8)

[a]Mean (SD)

Table 2: Associated factors between postoperative infection rate in vancomycin graft soaking with vancomycin group and non-soaking group in primary ACLR using simple logistic regression (n=74)

Variable	OR(95% CI)	Wald statistics	P-value
Age	0.976(0.923,1.032)	0.732	0.392
Gender			
Male	1		
Female	1.276(0.294,5.542)	0.106	0.745
BMI(kg/m²)			
<30	1		
>30	0.605(0.149,2.464)	0.491	0.483
Smoking status			
No	1		
Yes	1.546(0.542,4.408)	0.664	0.415
Diagnosis			
ACL tear only	1		
ACL tear with meniscus tear	0.906(0.327,2.512)	0.036	0.850
Duration of surgery	1.003(0.987,1.019)	0.145	0.704
Postoperative Infection			
No	1		
Yes	0.181(0.019,1.707)	2.227	**0.136**

OR-Odds Ratio; CI-Confidence Interval

Table 3: The comparison between postoperative infection rate inthe vancomycin graft soaking group and non-soakinggroup in primary ACLR

Variable	Group A n (%)	Group B n(%)	Total n(%)	P-value
Age	27.79(8.36)	26.12(8.35)	-	0.397[a]
Gender				
Male	29 (89.7)	37 (90.2)	66 (89.2)	0.516
Female	4 (12.1)	4 (9.8)	8 (10.8)	
BMI(kg/m²)				
<30	28 (84.8)	37 (90.2)	65 (87.8)	0.361
>30	5 (15.2)	4 (9.8)	9 (12.2)	
Smoking status				
No	23 (69.8)	32 (78.0)	55 (74.3)	0.290
Yes	10 (30.3)	9 (22.0)	19 (25.7)	
Diagnosis				
ACL tear only	9 (27.3)	12 (29.3)	21 (28.4)	0.530
ACL tear with meniscus tear	24 (72.4)	29 (70.7)	53 (71.6)	
Duration of surgery	130.70 (34.84)	133.29 (24.38)	-	0.708[a]
Postoperative Infection				
No	29 (87.9)	40 (97.1)	69 (93.2)	0.099
Yes	4 (12.1)	1 (2.4)	5 (6.8)	

[a]Mean(SD) reported; P-value was based on an independent t-test, Pearson Chi-square was applied.

4. Discussion

In this study, we found less rate of postoperative infection occurring in the vancomycin graft soaking group compared to the non-soaking group. Four infection cases out of 33 patients in Group A (12.12%) and one infection out of 41 patients occurred in Group B (2.44%), respectively. We also found two cases out of 74 patients (2.70%) to be deep SSI or septic arthritis, higher than the other centers worldwide.

From this study, we could learn that the vancomycin soaking practice did reduce the postoperative infection rate, as

Grayson et al. proposed [15]. However, the overall findings that supporting the use of vancomycin (presoaking of the hamstring graft) in combination with prophylactic IV antibiotics reduced the postoperative infection rate did not reach significance in this cohort.

Many confounding factors were essential in determining the incident following ACLR regarding surgical site infection. Apart from contamination, surgeons should ideally modulate both patient and surgical risk factors to reduce the incidence of postoperative infections. Diabetes Mellitus was a significant risk factor (OR = 3.05) for SSI in a study of orthopaedic procedures in an ambulatory surgery center [18].Preoperative smoking habit is also associated with an increased risk of infection [19]. Smoking has been suggested to contribute to disease by delaying wound healing through nicotine-mediated vasoconstriction, causing endothelial dysfunction and impaired systemic immune response. There were 19 patients (25.7%) who were smokers identified. In this study, smoking increased the odds of infection by 1.5 times but did not reach statistical significance (95% CI = 0.542 to 4.408; p=0.415).

Maletis et al. determined an 8% increased risk of superficial infections per unit increase in BMI, which they postulated due to increased soft tissue dissection required for autograft harvest in patients with an increased BMI [4].Brophy et al. reported that patients with diabetes undergoing ACL reconstruction have a significantly elevated risk of postoperative infection (18.8-times higher odds) compared with those patients without diabetes but did not find any association of age factor and BMI with the risk of infection after ACL reconstruction [20]. The other meta-analysis reported that patient risk factors such as male gender, obesity of Body Mass Index (BMI) > 30kg/m², smoking, history of steroid administration, depression, rheumatoid arthritis, and diabetes mellitus are at increased risk of postoperative infection [12]. There were only nine patients (12.2%) who were obese with Body Mass Index (BMI) > 30kg/m². However, our analysis results were not supported by the findings observed in the previous studies (OR=0.605, 95% CI=0.149 to 2.464; p=0.483).

Decreased operative time was associated with a lower risk of infection following knee arthroplasty, or objectively, operative time of longer than 210 minutes, compared with less than 120 minutes, was associated with an increased risk of infection [21]. In their sub-analysis of operative time, the same author also concluded that each 15-minute increase in operative time was associated with a 9% increase in the risk of deep surgical site infection. Our study found that the mean duration of surgery was 2.2 hours but did not have statistical significance concerning postoperative infection (p=7.08). Overall, we could not find any statistically significant association of risk factors of smoking, high BMI, and prolonged duration of surgery with postoperative infection in the analysis.

The hamstring autografts were associated with a higher incidence of infection than other graft types, as reported by many studies [2,4,6].Previous knee surgery and concomitant open surgical procedures were often, but not universally, found to be associated with postoperative infections [2,3]. Viola et al. also reported tibial fixation with a metallic post and washer, a post–braided suture construct, and femoral-side Endo Button fixation was associated with an increased risk of infection [3]. Furthermore, additional procedures during ACL reconstruction could be risk factors for infection because of increased operative time, other incisions, or increased foreign body load [6].

Many centers worldwide have practiced the intra-operative autologous hamstring graft soaking with vancomycin antibiotics in primary ACLR to reduce the risk of postoperative infection. Vancomycin is a potent bactericidal antibiotic to eradicate *Staphylococcus* and *Enterococcus* bacterial infections [13]. It is reportedly less toxic to eukaryotic cells than the cefazolin or aminoglycosides group, and it has been shown to have thermostable properties [16]. The practice of vancomycin graft soaking is additional prophylaxis, along with the use of a preoperative intravenous prophylactic antibiotic which has demonstrated efficiency in reducing the postoperative infection rate [13]. For many years vancomycin has already been utilized in both local prevention and treatment in various circumstances in which it is reported to be safe for local use. For example, in clinical settings, vancomycin has been mixed with cement spacers, bioactive glasses, or composite biomaterials [17]. The hamstring autograft presoaking with vancomycin has shown promising results in decreasing the infection rate following an ACLR [12]. The minimal required concentration is about 2µg/ml to eliminate most *Staphylococcus* infections. The same elution was lower than the reported osteoblast and chondroblast toxicity concentrations [15]. Grayson et al. reported that a vancomycin solution presoaked into a tendon graft can sustain this minimal concentration for at least 24 hours for its effect [15].

In this present investigation, our findings were different from other pilot studies. They could be attributed to the small numbers of the population in the study, as well as inhomogeneity between the two groups for comparison.We found some limitations and weaknesses in this study. It was a retrospective study. This study enrolled a relatively small sample size (n=74). A bigger sample size may have a higher probability of postoperative infection. Besides, there was possible unintentional bias in patient selection, including the inclusion and exclusion criteria inherent to the nature of the study. Therefore, a prospective multicenter randomized control trial may provide more robust evidence.

5. Conclusion

The additional technique of hamstring autograft soaking with vancomycin solution compared to a single prophylactic IV antibiotic could reduce the postoperative infection rate after primary ACLR. Antibiotic graft soaking may be relevant as an additional prophylactic measure to prevent infection after ACLR.

Disclosure

The authors declare they have no conflict of interest.The authors did not receive any funding or grants in support of their research for this study.This study complied with ethical principles outlined in the Declaration of Helsinki and the

Malaysian Good Clinical Practice Guidelines. It involved no potentially vulnerable subjects. This study obtained ethical clearance from the Medical Research & Ethics Committee (MREC) before the commencement of this study.

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