Histological and immunohistochemical evaluation of human saphenous vein harvested by endoscopic and open conventional methods

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Abstract

OBJECTIVES: The introduction of endoscopic saphenous vein harvesting (ESVH) has been reported to decrease saphenectomy-associated wound pain and infection, compared with the traditional open conventional saphenous vein harvesting (OCSVG) technique. Despite these benefits, the rate of adoption among surgeons has been variable. Criticism of this technique centres on the risk of injury at the time of vein harvest with its potential detrimental effect on structural viability and long-term patency. The aim of our study is to investigate the endothelial preservation of saphenous vein grafts harvested by various extraction methods.

METHODS: A prospective, observational study of 30 human saphenous vein grafts was performed to evaluate endothelial preservation by haematoxylin–eosin and CD 31 staining methods. The saphenous vein was harvested endoscopically either by an open tunnel (OT-ESVH), closed tunnel (CT-ESVH) or an OCSVH harvesting technique. Research samples were collected without distension to avoid intraluminal dilatation and endothelial disruption. Both haematoxylin–eosin and immunohistochemistry slides were imaged by a high-resolution slide-scanning system.

RESULTS: Haematoxylin–eosin staining of the CT-ESVH group showed mostly preserved endothelium ($P = 0.398$) with some endothelial stretching ($P = 1.0$) and no endothelial detachment ($P = 0.197$). The OT-ESVH group showed marked endothelial stretching ($P = 0.053$). However, the OCSVH group showed significantly more endothelial detachment than the endoscopic groups ($P = 0.011$). The mean grading score of immunohistochemistry using the CD 31 antibody was much lower in the OT-ESVH group ($1.6 \pm 0.84$, $P = 0.009$), showing more poorly preserved endothelial cells than the CT-ESVH and OCSVH groups.

CONCLUSIONS: We observed more endothelial stretching in the OT-ESVH group, which in our opinion, was due to lack of subcutaneous tissue separation, poor visualization and traction stresses across the wall of the saphenous vein. However, the OCSVH method revealed poor endothelial protection with areas of endothelial detachment, not observed with both endoscopic techniques. Interestingly, most preserved endothelium was found in the CT-ESVH group, which was previously known to be associated with worse graft patency.

Keywords: Human saphenous vein • Endoscopic vein harvesting • Open conventional vein harvesting

INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of myocardial infarction and sudden cardiac death in the industrialized world [1]. Although mortality from CAD continues to decline in several areas of the world [2] including most countries of Europe, America and Australia, unfavourable trends were still observed in the Russian Federation and other countries of the former Soviet Union, whose recent rates remain exceedingly high [3].

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the SV has become the most commonly used conduit in patients undergoing coronary artery bypass grafting (CABG). However, poorer long-term patency rates and clinical outcomes have been reported after CABG performed using SV grafts compared with those after CABG with arterial grafts, such as the internal thoracic artery and right gastroepiploic artery [8]. Our aim is to investigate the histological and immunohistochemical findings of the saphenous vein graft, to rule out endothelial damage as a direct result of manipulation or instrumentation by endoscopic and open conventional harvesting methods.

**MATERIALS AND METHODS**

**Study hypothesis and aims**

Mechanical traction and manipulation during endoscopic harvest of saphenous vein grafts may cause structural damage and could result in impaired endothelial function.

**Aim 1: histological analysis.** To assess the structural viability of saphenous vein endothelium by light microscopy.

**Strategy:** To perform haematoxylin–eosin staining of human saphenous vein sections and assess endothelial viability.

**Aim 2: immunohistochemical analysis.** To assess the structural viability of saphenous vein endothelium by immunohistochemistry.

**Strategy:** Staining human saphenous vein with the CD 31 antibody and assessing the degree of endothelial expression.

**STUDY DESIGN**

We prospectively analysed 30 human saphenous vein samples from three different treatment groups for this study. The patients were allocated a harvesting technique at the time of listing without any selection bias. Allocations were independent of any patient-related factors such as age, sex, weight, diabetes, peripheral vascular disease etc.

The study protocol was approved by the institutional ethics committee and informed consent was obtained from all study patients. Storage and handling of human tissue was covered by the Human Tissue Act licence held by the institution. Labelling, imaging and image analysis were all done in a blinded fashion. Histology and staining was performed by a Shandon Varistain 2255 fully automatic rotary microtome. Haematoxylin and eosin staining was performed by the research lab for storage in liquid nitrogen until further use. After thawing for 10 min at 37°C, sectioned vein specimens enclosed in labelled tissue processing cassettes were fixed in freshly prepared neutral buffered formalin. This is 10% formalin buffered with sodium dihydrogen phosphate and disodium hydrogen phosphate to pH 7.0, i.e. with Sorenson’s buffer. Using this solution ensures that the pH of the fixative remains constant before and during fixation. Bromothymol blue was added to the fixative, as a bluish green pH indicator. The same protocol was followed for all three groups without any significant time difference between harvesting, storage and processing (~48 h).

**Histology and staining**

All paraffin embedded samples were sectioned at 5 mm by a Leica 2255 fully automatic rotary microtome. Haematoxylin and eosin staining was performed by a Shandon Varistain™ 24-4 automatic slide stainer to evaluate endothelial preservation. Histologically endothelial viability was classified into three categories: normal endothelium (Grade 0), stretched endothelium (Grade 1) and detached endothelium (Grade 2).

**Immunohistochemistry**

CD 31 staining was performed using mouse monoclonal antibodies against human endothelial glycoprotein CD31 (1:25, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Endogenous peroxidase activity of the sections was blocked by 10-min preincubation with 3% hydrogen peroxide in methanol. Sections were then incubated in 5% normal goat serum in Tris buffer solution for 1 h. After washing, sections were incubated in 5% normal goat serum in Tris buffer. Using this solution ensures that the pH of the fixative remains constant before and during fixation. Bromothymol blue was added to the fixative, as a bluish green pH indicator. The same protocol was followed for all three groups without any significant time difference between harvesting, storage and processing (~48 h).
used the refined scoring system used for immunohistochemical staining [9, 10]. The grading system was dependent on the uptake of staining along the circumference of whole intimal layer. We slightly modified the existing grading system for simplicity. No staining (Grade 1), trace (Grade 2), minimal (Grade 3), moderate (Grade 4) and marked (Grade 5).

Image processing

Both haematoxylin–eosin and immunohistochemistry slides were imaged by Pannoramic 250™ slide scanning system. It has high-NA Carl Zeiss™ optics to achieve maximum resolution of up to 0.16 µm per pixel. Scanned images were sent directly to the University of Manchester Bioimaging server for immediate sharing and analysis. The Pannoramic Viewer™ software was used for efficient image viewing, annotation and archiving.

STATISTICAL ANALYSIS

Statistical package for the social sciences (SPSS) version 20.0 was used for data analysis. Data were expressed as mean ± standard deviation (SD) or proportions. We used one-way analysis of variance to compare the statistical significance between mean values of the three groups. For all analyses, a P-value of ≤0.05 was considered statistically significant.

RESULTS

Histological analysis

The CT-ESVH group showed mostly preserved endothelium (P = 0.398; Fig. 1A, B and D) with some endothelial stretching (P = 1.0; Fig. 1C) and no endothelial detachment (P = 0.197). Similarly, OT-ESVH group showed normal endothelium (P = 0.398; Fig. 2A and B) with more endothelial stretching (P = 0.053; Fig. 2C and D) and no endothelial detachment (P = 0.197). However, the OCSVH group showed more endothelial detachment than the other groups (P = 0.01; Fig. 3B) with normal endothelium in most sections (P = 1.0; Fig. 3A, C and D) and no endothelial stretching (P = 0.073). Bar chart shows endothelial viability (haematoxylin–eosin staining) by various extraction methods (Fig. 4).

Immunohistochemical analysis

Immunohistochemistry was performed using antibodies against human endothelial glycoprotein CD31, to assess the degree of staining in the intimal layer. The mean grading score of immunohistochemistry using the CD31 antibody was much lower in the OT-ESVH group (1.6 ± 0.84, P = 0.009, Figs 5 and 6), showing poorly preserved endothelial cells (ECs), than the CT-ESVH and OCSVH groups. CD31 antibody staining in the CT-ESVH group (3.2 ± 1.23, P = 0.03, Figs 5 and 7) showed better endothelial protection than the rest of the groups and especially when compared with the OT-ESVH group. The OCSVH group (2.6 ± 1.43, P = 0.70; Figs 5 and 8) also showed better endothelial preservation than the OT-ESVH group, but worse than the CT-ESVH group.

DISCUSSION

Our results showed better endothelial preservation with CT-ESVH technique when compared with the OT-ESVH method. However, the OCSVH method revealed poor endothelial protection with areas of endothelial detachment, not observed with both endoscopic methods. Our surgical strategy was to avoid pressure distension of vein grafts, to prevent stretching of the vein wall and vascular smooth muscle cells. This enabled us to investigate the true effect of CO2 and mechanical trauma inflicted on saphenous vein grafts during harvesting.

ESVH was introduced into cardiac surgical practice to eliminate the need for long skin incisions, and it was associated with fewer

Figure 1: Haematoxylin–eosin staining of the CT-ESVH group showing normal endothelium, Grade 0 (A, B, D), and endothelial stretching (black arrows), Grade 1 (C).
wound complications, shorter hospital stays, less postoperative pain and better patient satisfaction [11]. A randomized trial ($n = 200$) comparing the 6-month patency rate and leg wound complications of endoscopic with open vein harvest, showed reduced leg wound complications in the endoscopic group without compromising the 6-month patency rate [12]. However, a landmark study [13] showed endoscopic vein-graft harvesting independently associated with graft failure and adverse clinical outcomes in a secondary analysis of 3000 patients undergoing CABG (endoscopic: 1753 patients vs open harvesting: 1247 patients). This was a significant finding, resulting in a debate and apprehension among surgeons to use endoscopically harvested vein grafts for coronary artery surgery.

Moreover, CO2 insufflation used by many endoscopic vein extraction systems facilitates dissection by creating a subcutaneous tunnel. Normally, the insufflated CO2 is absorbed by the blood and must be eliminated by the lungs through increased ventilation [14]. In the literature, some cases of CO2 embolisms or hypercarbia during this procedure are described [15, 16]. The mechanism of CO2 embolism during endoscopic procedures is supposed to be CO2 absorption or its direct entry, through an injured vessel, into the blood stream.

In our study, we observed more endothelial stretching, without any detachment in the OT-ESVH group, which, in our opinion, was due to lack of tissue separation, poor visualization and traction stresses across the wall of the saphenous vein and its side branches.

Figure 2: Haematoxylin–eosin staining of the OT-ESVH group showing normal endothelium, Grade 0 (A and B), and endothelial stretching (black arrows), Grade 1 (C and D).

Figure 3: Haematoxylin–eosin staining of the OCSVH group showing normal endothelium, Grade 0 (A, C and D), and detached endothelium (black arrows), Grade 2 (B).
A mechanism was reported in the literature by two studies [17, 18] to involve a local vasodilatory effect and prolong graft survival. This mechanism is known to promote a true vasomotor function, especially calcium mobilization and eNOS-related NO production. These properties are known to promote a local vasodilatory effect and prolong graft survival. This protective mechanism was reported in the literature by two studies [17, 18] but they fail to establish the link between CO2 insufflation and attenuated NO release from the endothelium and the adventitial Vasa Vasorum during endoscopic extraction. We hypothesized that abundance of CO2 in the subcutaneous tunnel causes this inhibitory effect, which we will try to prove in the next phase of our study.

Both endoscopic methods commonly require bipolar cautery in the vicinity of vein grafts, whereas neither insufflation nor bipolar energy is required for the OCSVH technique. The use of cautery has been proposed to cause thermal injury to the vessel wall, which may impair graft quality by compromising the viability of ECs, resulting in platelet aggregation and thrombosis. Chronic endothelial damage and dysfunction stimulate migration and proliferation of smooth muscle cells into the intima, a key event in the development of atherosclerosis and SV graft failure [19]. Variable CO2 pressure in the OT-ESVH method might have exposed the vein graft to traction-induced trauma and vessel spasm. The variability in the intraluminal flow from extrinsic pressure fluctuations may have resulted in increased shear stress and damage to the luminal endothelium. Although both endoscopic methods have similar harvesting times, we found the OT-ESVH method technically more challenging due to lack of optimal visualization. We have used the OCSVH method of harvesting, in which a vein is isolated from its surrounding connective tissue and even in most experienced hands results in trauma and vessel spasm. Recent studies have demonstrated histological differences between conventionally harvested SVs and those harvested using a no-touch surgical technique [20]. The perivascular fat and adventitia (outer vessel wall layer) are generally stripped or damaged during vein harvesting. This plays an important role in graft dysfunction because perivascular fat and adventitia do not merely provide structural support for the media, but also contain a microvascular network responsible for the exchange of gases and supply of nutrients to the vein wall. Periadventitial fat-derived nitric oxide has proved to play a beneficial role in improved SV patency harvestedatraumatically by a no-touch technique [21].

The mean grading score of immunohistochemistry using the CD31 antibody was much lower in the OT-ESVH group, showing poorly preserved ECs, than the rest of the groups. The OCSVH group showed better endothelial preservation than the CT-ESVH group, but worse than the CT-ESVH group, although this was not statistically significant.

In our study, among the endoscopic groups, the CT-ESVH method showed the highest CD31 antibody grading scores, with 50% more staining in the intimal layer. ECs constitute not only a barrier between blood and extracellular matrix but also a source of molecules that influence both the structural and functional integrity of vessel wall permeability and circulation. They secrete a wide spectrum of molecules into the blood as well as to the subendothelial extracellular matrix. These molecules are involved in the formation of platelet and fibrin thrombi [e.g. von Willebrand factor (vWF) and tissue factors], contribute to antithrombotic properties of the endothelium (e.g. prostacyclin, thrombomodulin and heparan sulphate), express plasminogen activators and inhibitors, regulate growth of other cells, bind lipoproteins and hormones and are involved in immune reactions [22]. CD31 is found in large amounts on ECs and is less abundant on platelets and most leucocytes. It plays a major role in a number of cellular interactions, most notably in the adhesion cascade between ECs and polymorphonuclear leucocytes, monocytes and lymphocytes (by heterophilic cell interactions) in inflammatory processes and between adjacent ECs (by homophilic cell interactions) during the process of angiogenesis [23].

Figure 4: Bar chart representing endothelial viability by various extraction methods. Endothelial stretching was observed in the OT-ESVH group (P = 0.05), whereas endothelial detachment was present in the OCSVH group (P = 0.01).

Figure 5: Bar chart representing CD31 staining by various extraction methods. The OT-ESVH group showed poor expression of the CD31 antibody (P = 0.009) than the CT-ESVH group (P = 0.03).
CD31 is one of the most commonly used endothelial marker for assessing endothelial structural integrity. Previous studies have reported that CD31 was strongly and homogeneously expressed in pulmonary vascular endothelial tissue [24]. Another study reported that the expression of EC markers CD31, CD34 and vWF in the vascular tree was heterogeneous, with specific patterns for individual vessel types and different anatomical compartments of the same organ [15]. The staining pattern of large veins like the inferior vena, pulmonary and femoral vein showed continuous and strong staining for vWF. The staining pattern was usually discontinuous for CD31 and CD34, the intensity being less for vWF. CD34 also stained some components of the media, probably corresponding to smooth muscle cells [25]. There are limited data available on the immunohistochemical expression pattern of CD31 in saphenous vein endothelium and we observed a similar discontinuous staining pattern of ECs and smooth muscle cells in our study. Poor endothelial preservation reflected by weak CD31 staining in our OT-ESVH group supports the idea of traction-induced injury by endoscopic manipulation. Similar results were observed by haematoxylin–eosin staining from the same group, showing endothelial stretching.

Figure 6: CD31 staining of the OT-ESVH group: (A) trace (Grade 2), (C) minimal (Grade 3), (B, D) no staining (Grade 1). CD31 was stained brown in colour.

Figure 7: CD31 staining of the CT-ESVH group: (A) marked (Grade 5, black arrows), (B) moderate (Grade 4) with staining of the whole intima (black arrows), (C) minimal (Grade 3) and (D) trace (Grade 2). CD31 was stained brown in colour.
These histological findings should be interpreted with caution, as we have examined only the histological features and staining patterns of the vein, which may not reflect the true effects of CO₂ insufflation and thermal damage on the vasomotor function of the SV graft, which need to be further investigated. We believe that damage to the segments of the saphenous vein harvested either by open or an endoscopic method is inevitable, but by mastering less traumatic harvesting techniques, improved graft patency and function may be achieved.

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**REFERENCES**


