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## Evaluation of Vascular Access Using Cryopreserved Jugular Vein (Experimental Study)

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### ABSTRACT

**Background:** While considering the rise in the mean age of the chronic renal failure (CRF) patients and increasing frequency of those who need constant hemodialysis, the creation of a native arteriovenous fistula (AVF) is not possible, which could be due to the inaccessibility of the superficial veins. This study was conducted to evaluate the efficacy of the vascular access, using cryopreserved jugular vein.

**Materials and Methods:** In this experimental study, 15 sheep (Wt. = 30-40 kg) were selected between the year 2001 and 2002. The external jugular vein of each sheep was excised and after cryopreservation was put in liquid nitrogen (-196°C). Twenty-eight days later, the sample veins were used as allograft (in another sheep), and autograft (in the same sheep) and were placed between carotid artery and external jugular vein as bridge AVF. The efficacy, function, and patency were evaluated using doppler sonography and pathologic report.

**Results:** Patency rate of sample was 100% after 3 months in both allografts and autografts. Thrombosis, hematoma, and infection were not seen. No inflammation was detected in pathologic report.

**Conclusion:** This study showed that using allograft vein for vascular access is feasible and suitable, and it is recommended specially for those with previously infected fistula. (*Tanaffos* 2004; 3(10): 13-17)

**Key words:** Arterio-venous fistula (AVF), Cryopreservation, Vascular access

### INTRODUCTION

During the past two decades, the number of patients under chronic hemodialysis has significantly increased (1), so that angioaccess is considered as one of the most common surgical procedures on vessels (1-2). In most of the patients due to inaccessibility of the superficial veins, creation of a native AVF is not possible (2,3).

Thus, the most common substitute is a synthetic prosthesis like polytetrafluoroethylene (ePTFE) which can act as a bridge between artery and vein for creating fistula (bridge AVF) (2,3). Despite the precedence of ePTFE, the patency of created fistulas, even the bovine heterograft type is more than ePTFE (4,5). Infection is one of the most important failures of ePTFE with a frequency that has been estimated between 11% and 35% (6,7). It is notable that in the

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presence of infection, even at sites far away from the vascular sutures, conservative treatment for the control of sepsis and patency fails and graft removal would be almost always required (6,7). Furthermore, the possibility of pseudo-aneurysm formation is high because of fibrosis and repeated needle punctures (8).

The appropriate choice is finding a substitution as a live tissue that not only inhibits platelet aggregation but also has tissue repair and risk of infection that is similar to other living tissues (9, 10). Numerous studies have been performed using live tissues as substitution tissues; the most common limitation is the possibility of graft rejection by immune mechanisms (6,7). Cryopreservation may reduce antigenicity. This study was conducted with the aim of evaluating the vascular access by using sheep's cryopreserved jugular vein.

## **MATERIALS AND METHODS**

This experimental study was performed in animal laboratory of Massih Daneshvari hospital between the year 2001 and 2002. Fifteen sheep (W= 30-40 kg) were selected in a non-randomized sampling.

All of them were kept at the same conditions (i.e food, light, temperature and water). Twelve out of 15 sheep underwent general anesthesia by ketalar 20 mg/kg IM and acepromazine 0.4 cc/kg IM, and subsequently by halothane and ketalar 20 mg/kg IM. Right jugular vein was excised, heparinized (150 IU/kg), frozen, and sent for further evaluation. The excision of jugular vein was as follows: longitudinal incision was given on the anterior part of the sternocleidomastoid muscle; external jugular vein was exposed and ligated at the both ends.

For cryopreservation, jugular veins were washed by UW solution containing allopurinol at 4°C. Then it was put in DMEM (Dulbeco's Modified Eagle Medium) containing DMSO 10% (dimethyl sulfoxide), FCS 20% (Fetal calf serum) and 5%

trihalose for 4h at 4°C. Later the samples were cooled to -60°C for 60 min (1°C/min with MLW cryopreserver), then the samples were immediately put in liquid nitrogen. Twenty-eight days later, the samples were brought out from liquid nitrogen (for allograft and autograft) and immediately placed in water bath (T=39°C) and returned to normal temperature.

After prep. and drep., the external jugular vein was excised, under general anesthesia, with longitudinal incision given on the anterior part of sternocleidomastoid muscle. The carotid sheath was opened and heparin was injected through it. The cryopreserved vein (length: 5-8 cm) was anastomosed by proline suture (7/0) in the "end to side" form to the carotid artery, at a distance of 2.5 cm from bifurcation (superior one-third of the neck). The distal end of cryopreserved vein was anastomosed as "end to side" to external jugular vein with the same suture (inferior one-third of the neck). Therefore, blood flow in the shunt was from up to down.

The opposite external jugular vein was ligated too. All of these stages were done for 12 sheep as autograft. In remaining 3, the vascular access was also placed in with the same procedure with the exception that allograft veins were used.

The cases were divided randomly into three groups (each group had 5 sheep). The procedures were performed at different times for each group. On days 7, 21 and 90 the shunt, left carotid artery and Jugular vein were brought out as "enblock", under general anesthesia and sent for pathological evaluation.

The necessary informations like weight, presence of thrill, hematoma and, infection, the state of rejection (clinically, by presence of thrill and pathologically, by lymphocyte rejection) were recorded on prepared questionnaires. In each group,

autograft and allograft methods were performed on 1 and 4 sheep, respectively.

## RESULTS

No bleeding was detected from wall of the shunt and the site of anastomosis. Immediately after anastomosis there was a palpable thrill in all shunts except in one. In this case the thrill was detected after 24 hr. No hematoma was found in samples. During the follow up, thrill was felt on the sheep's neck and was present until 6 months after surgery.

Doppler sonography of shunt showed appropriate flow without obstruction and thrombosis. There was no vortex circulation in the shunt on days 21 and 90. In addition, the vein was patent and blood flow was evident. Pathologically, the 3 autografts showed no lymphocytes infiltration in the intact intima, media, and adventitia layers on days 21 and 90. Meanwhile microscopic evaluation of 12 allografts on days 7, 21, 90 showed that intima, media, and adventitia layers of the cryopreserved vein were similar to that of jugular vein and did not have lymphocyte infiltration. Electron microscope also confirmed these findings.

## DISCUSSION

In this research, the grafts were removed on days 7, 21 and 90 after surgery. Macroscopically, color change and thrombosis were not detected. Additionally, no difference was seen between cryopreserved vein and external jugular vein either before or after surgery (on days 7, 21 and 90).

Microscopically, there was no evidence of inflammation and cellular infiltration on venous side of allograft vein where blood flow is slow and the risk of thrombosis is high. This indicates that there will be no evidence of rejection at least 3 months after surgery.

Similar studies like Ruddle's study have shown that vascular smooth muscles are slightly damaged

during the cryopreservation. They had also used DMSO as antifreezing material and demonstrated that in the absence of DMSO there is destruction of cells during the thawing stage causing tissue damage (5).

In this study, the patency rate of allograft vein on macroscopic examination and doppler sonography was 100% during 6 months after surgery. This is a high rate in comparison with other studies like Castier's study (France) in which cryopreserved arteries had been used for re-vascularization of injured limb vessels; the patency rate was 75% during the 6-month period (10).

This difference may be due to larger diameter of external jugular vein of sheep (in our study) in comparison with femoral and popliteal cryopreserved allograft arteries (in Castier's study). The jugular vein with its large diameter can prevent thrombosis. On the other hand, allograft cryopreserved veins function better as compared to cryopreserved arteries in the long range. It is recommended to study the patency rates of different vessels with different diameters.

It must be noted that large diameter of the vein can expose vascular endothelium to larger number of circulating antigens indicating good efficacy of allograft veins.

In this study, the temperature of cryopreserved vein reached to  $-196^{\circ}\text{C}$  vs.  $-80^{\circ}\text{C}$  in Castier's study. This may cause low antigenicity and consequently higher patency of allograft veins in our study. On the other hand, high pressure difference between carotid artery and external jugular vein may be another cause of high patency observed in this survey.

We did not detect any surgical infection in 15 grafts. The sheep had received prophylactic antibiotic during anesthetic induction. The site of surgery was dressed with chloramphenicol spray. After 3 months, no sign of infection was found at surgical site. The resistance of allograft vein against infection has also

been observed in other studies. In a study performed by Matsuura et al., femoral allograft veins were used as grafts for bridge AVF. In 82% of cases, the reason for using cryopreserved veins was the infection of previous vascular accesses (using ePTFE graft). Some cases had sepsis in addition to local infection. Using cryopreserved allograft veins had two advantages:

- 1- Appropriate 6-month patency (75%) which was comparable with that of ePTFE graft (78%).
- 2- Infection was controlled (and sepsis was improved). They used injectable antibiotics 2 weeks before grafting; *Staphylococcus aureus* was found at the site of surgery (1).

## CONCLUSION

Regarding the clinical and pathologic findings of the study samples and also the efficacy of cryopreserved veins (shown in numerous studies), for either replacing vascular shunts (for hemodialysis) or revascularization (for saving the limb), we can conclude that cryopreservation is practical in human models. In addition, it will be useful for short term specially in infectious fields. It is notable that since pathologic changes can occur in the grafted veins in the long run, pathologic evaluations of shunts should be performed from 6 months to one year time and continued until long- term use of graft is confirmed.

We also suggest to assess and evaluate factors that affect the efficacy of shunts.

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