Functional Evaluation of Sciatic Nerve Anastomosis Wrapped by Freeze Dried Human Amniotic Membrane in Sprague Dawley Rat

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Abstract

Background. Peripheral nerve injury (PNI) is a common medical condition. The defected nerve, if not repaired as early as possible, can cause long-term denervation and neurotrophic failure for the target organ. This leads to a series of denervation manifestations, such as muscle atrophy, loss of sensory function, etc. and ultimately, these manifestations seriously affect the patient’s sensorimotor function.¹,² Amniotic membranes have been widely used in ophthalmology and skin injury repair because of their anti-inflammatory properties. Objective: In this study, we measured therapeutic efficacy and determined if amniotic membranes could be used for sciatic nerve repair. Methods. A posttest only control group design has been done in 10 healthy Sprague Dawley rats. In all rats, a unilateral rightside sciatic nerve transection was performed and reanastomosed by different methods: Group I (control group): included 5 rats, the anastomosis was done by epineural microsutures using 8/0 nylon. Group II: included 5 rats, the anastomosis was done by epineural microsutures using 8/0 nylon and then wrapped by freeze dried human amniotic membrane. Functional evaluation of nerve recovery was done over 3 weeks postoperatively using walking tract analysis and calculate using Sciatic Functional Index. Result. Functional results showed that there was no significant difference of the sciatic functional index (SFI) between group I and group II. Conclusion. We can conclude that during 3 weeks functional evaluation, there is no significant difference between control group and experimental group that achieved freeze dried human amniotic membrane. Keywords: sciatic nerve injury, freeze dried human amniotic membrane, walking tract analysis, sciatic functional index.

Abstrak

I. INTRODUCTION

Peripheral nerve injuries (PNI) are common and have marked impact on the everyday life of the population at large. Thirty percent of these injuries arise from lacerations by sharp objects and long bone fractures, and in the remaining penetrating injuries, crush, ischemia, traction, electric shock and vibration play a role. Approximately 100,000 patients undergo peripheral nerve surgery in the USA and Europe annually. Severe nerve injury has a devastating impact on patient’s quality of life.

Synkinesis and axonal misrouting are the common complications after facial nerve repair or nerve anastomosis. Thus, it is essential to choose a suitable surgical modality to provide satisfactory results considering both aesthetics and functionality. The surgical modalities may be nerve grafts, regional muscle transfers, primary neurorrhaphy (anastomosis), free tissue transfers, static procedures, and nerve transfers. The gold standard technique is primary neurorrhaphy, but there is a long delay period between the injuries and repair. Nerve grafts, regional muscle transfers, and free tissue transfers are the preferred alternative techniques.

Amniotic membrane (AM) may be a good candidate to solve this problem. The amniotic membrane is an avascular membrane that is composed of an epithelial layer and an inner mesodermal tissue, which can reduce potent proinflammatory cytokines. AM has been widely used in ocular reconstructions, wound care, etc. It has been used as fresh AM, dehydrated AM, and freeze-dried AM. Nakamura et al (2014) concluded that the sterilized, freeze-dried AM retained most of the physical, biological, and morphologic characteristics of cryopreserved AM.

This study will see the effect of freeze-dried amniotic membrane on nerve regeneration after anastomosis of sciatic nerve on Sprague dawley rat, based on functional evaluation using walking track analysis.

II. METHODS

This is an experimental study, post test only control group design. This study was held at Klinik LPPT Gadjah Mada University on 1st – 22nd August 2015. Subject of the study was Sprague Dawley rat, male, 3 m.o, 250 g weight. Ten rats were divided into 2 groups; Group I (control group): right sciatic nerve was cut by surgical blade no 11, then anastomosed using monofilament non-absorbable no 8/0; Group II (experimental group): right sciatic nerve was cut by surgical blade no 11, anastomosed using monofilament non-absorbable no 8/0, and then wrapped by freeze dried human amniotic membrane.

Walking track analysis (WTA) was used to evaluate the nerve regeneration on day -1, 7, 14, and 21. From data that was gained from WTA then we calculated the Sciatic functional index (SFI) using the formula:

$$SFI = 38.3 \times \frac{EPL-NPL}{NPL} + 109.5 \times \frac{ETS-NTS}{NTS} + 13.3 \times \frac{FIT-NIT}{NIT} - 8.8$$

III. RESULT

All rats underwent walking track analysis at day 1, 7, 14 and 21. Both feet were smeared by ink and then placed on walking track box, as seen in the picture.

Figure 1. Both feet were smeared
As the rat walk on the walking track box, the foot printed was gained as seen at the picture bellow.

Walking tract analysis was counted from the foot print that was gained at the walking track (Sarikcioglu et al. 2008) as seen in the picture bellow:

The data that was gained from walking track analysis are presented on the following table below as mean of each day:

<table>
<thead>
<tr>
<th>Table 1. Mean Of Walking Track Analysis Of Each Day For Each Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
</tr>
<tr>
<td><strong>D1</strong></td>
</tr>
<tr>
<td>EPL</td>
</tr>
<tr>
<td>NPL</td>
</tr>
<tr>
<td>ETS</td>
</tr>
<tr>
<td>NTS</td>
</tr>
<tr>
<td>EIT</td>
</tr>
<tr>
<td>NIT</td>
</tr>
<tr>
<td>TOF</td>
</tr>
</tbody>
</table>

Sciatic nerve functional index was calculated using the formula that has been explained previously. The results are presented on the following table below:
An SFI of 0 indicates normal, and −100 indicates total impairment. However, de Medinaceli et al (1984)\(^7\) reported the normal values between +11 and −11. At this study we found that the SFI of all rats are ∼88, it indicates poor function. This value persists until day 21, as seen on the graphic bellow:

**Graphic 1.** Comparison of Average of SFI Group I and II on day 1, 7, 14 and 21.

From the graphic above we can see that there was no improvoment at all, the SFI persists at value of ∼88 until day 21 on both groups. T-Test result also shows there is no significant difference between control and experimental group for each day, as seen on the following table below:

**Table 2. Sciatic Nerve Functional Index (SFI) Of Each Rat In Control (Group I) And Experimental (Group II) Group**

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I (Mean ± SD)</th>
<th>Group II (Mean ± SD)</th>
<th>p (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1</td>
<td>D7</td>
<td>D14</td>
</tr>
<tr>
<td>&quot;D&quot;</td>
<td>&quot;D&quot;</td>
<td>&quot;D&quot;</td>
<td>&quot;D&quot;</td>
</tr>
<tr>
<td>Rat I</td>
<td>-88.91 ± 88.91</td>
<td>-88.91 ± 88.91</td>
<td>-88.91 ± 88.91</td>
</tr>
<tr>
<td>Rat II</td>
<td>-88.89 ± 88.89</td>
<td>-88.89 ± 88.89</td>
<td>-88.89 ± 88.89</td>
</tr>
<tr>
<td>Rat III</td>
<td>-88.90 ± 88.90</td>
<td>-88.90 ± 88.90</td>
<td>-88.90 ± 88.90</td>
</tr>
<tr>
<td>Rat IV</td>
<td>-88.92 ± 88.92</td>
<td>-88.92 ± 88.92</td>
<td>-88.92 ± 88.92</td>
</tr>
<tr>
<td>Rat V</td>
<td>-88.91 ± 88.91</td>
<td>-88.91 ± 88.91</td>
<td>-88.91 ± 88.91</td>
</tr>
<tr>
<td>Mean</td>
<td>-88.906 ± 88.906</td>
<td>-88.906 ± 88.906</td>
<td>-88.906 ± 88.906</td>
</tr>
</tbody>
</table>

**Table 3. T-Test Result Of SFI Control And Experimental Group For Each Day**

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I (Mean ± SD)</th>
<th>Group II (Mean ± SD)</th>
<th>p (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-88.906 ± 0.01140</td>
<td>-88.916 ± 0.01140</td>
<td>± 0.203</td>
</tr>
<tr>
<td>7</td>
<td>-88.906 ± 0.01140</td>
<td>-88.916 ± 0.01140</td>
<td>± 0.203</td>
</tr>
<tr>
<td>14</td>
<td>-88.906 ± 0.01140</td>
<td>-88.916 ± 0.01140</td>
<td>± 0.203</td>
</tr>
<tr>
<td>21</td>
<td>-88.906 ± 0.01140</td>
<td>-88.916 ± 0.01140</td>
<td>± 0.203</td>
</tr>
</tbody>
</table>

**IV. DISCUSSION**

Lately, research on the role of amniotic membrane in regenerative medicine being actively carried out. Karaman et al (2013)^4^ on the study said that the amniotic membrane is avascular membrane composed of epithelial cells and mesodermal tissue, which can reduce the potential proinflammatory cytokines. Besides being used in regenerative medicine, amniotic membrane has also been widely used as antiinflammatory effect on eye surgery, treatment of burns, surgery and wound closure. Amniotic membrane is also believed to have a low potential for adverse reactions. Tsai et al (2004)^8^ on the study said that only human amniotic membrane-derived mesenchymal stem cells that do not express molecules of major histocompatibility complex class I (MHC I). Maybe this is the cause of immunological tolerance is high.\(^8\)

Forbes J and Fetterolf DEC (2012)^9^ examines the use of amniotic membrane in the treatment of wounds and give good results. Roosenlbum B (2014)^10^ investigated the use of amniotic membrane allograft in diabetic ulcers no lower extremities. 5 ulcers from 3 patients were treated using amniotic membrane allograft after debridement, and the result is all wounds improved in the time range that is different.\(^10\)

Neural Regeneration Research Board\(^{11}\), USA has done some research on amniotic membrane. In one study the research agency said that the amniotic membrane epithelial cells expressing neuronal phenotypes (microtubule-associated protein-2, glial fibrillary acidic protein and nestin). Conditioned medium from human amniotic epithelial cells can trigger the growth and proliferation of rat glial cell cultures in vitro. Whereas in other studies the body of research proves the transformation of human amniotic epithelial cells into neuron-like cells in the microenvironment of head injury, in vivo and in vitro.\(^{11}\) Karatman et al (2013)^4^
showed that the amniotic membrane can help restore nerve injury fascialis.

The different results obtained in this study. Evaluation for 21 days showed no improvement in the group given amniotic membrane. This shows that within three weeks of the amniotic membrane has not proven to help restore the function of n. ischiadicus who suffered injury.

V. CONCLUSION

Limitations of this study is the evaluation of a short time, ie 3 weeks. Further studies with a longer time frame is needed to look at the role of amniotic membrane in the regeneration of peripheral nerve injury. Evaluation by other methods are also needed to get a more valid data. In addition to the evaluation function, the evaluation of nerve regeneration can also be done by making preparations for histopathology, electrophysiological evaluation, histomorfometri, or by measuring ankle angle. The combination of several methods of evaluation will produce data that is more valid and reliable.

References