EFFECT OF HYALURONIC ACID ON THE REGENERATION OF THE FACIAL NERVE IN DOGS: ELECTROPHYSIOLOGICAL AND NEUROHISTOPATHOLOGICAL STUDY

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Abstract
The aim of the study was to study the effect of Hyaluronic acid administration of one dose on regeneration of facial nerve injury. Eight adult healthy dogs are used, divided into two equal groups: Control Group and Hyaluronic Acid. Each all group includes four animals for histopathological study at 8 week and 12 weeks post operation. Facial Nerve by been crushed in the left thrusting face after the departure of the nerve of stylomastoid foramen and passes through the parotid gland which was divided into five main branches of the feeds and controls the muscles terms face and two - thirds of the inner part of the tongue on the process for taste and oral cavity then the skin sutured immediately by silk suture 0 - 3 mm with end – to - end anastomosis, using simple interrupted suture. After 48 hours, post operation. But, in the hyaluronic acid group, the dogs were injected with hyaluronic acid drug at a dosed 1 ml/20 kg B.W once a day’s post operation. The clinical findings for control group revealed the paralysis or weakness of the muscles of ears, eyelids, lips and nostrils. In the Hyaluronic acid group, the signs are similar to Control Group but the symptoms began after 5 days injection of acid and within two weeks, the animal seems normal. The laser surgery group has been exposed to the laser for 10 days and the symptoms begin gradually disappear. But, the neurohistopathological examination and macroscopic examination show that accelerated the regeneration of facial nerve and improvement of the motor and sensory function of the nerve was best in the Hyaluronic acid group. The advantage of Hyaluronic Acid accelerated regeneration of nerve and increase blood flow to the affected area to increase with nutrition.

Key words: Facial Nerve, Hyalournic Acid, Regeneration, Electrophysiological and Neurohistopathological.

1. Introduction
The search for effective tool for enhancing post traumatic nerve regeneration is one of the main challenges in Restored Cosmetic Medicine considering the high frequency of this type of injury (Alexander and Michael, 2012). Injury of peripheral nerve caused by traumatic events such as blast, gunshot or stab wounds, traction, contusion and compression injuries can cause several degrees of nerve injury (Bodner, 2009). The varied of injuries include paralysis sensory loss and not the disappearance of pain which may remain for months or years after injury (Mehdi et al., 2013). The facial nerve is one of a set of twelve cranial nerves originating from each side of the brainstem (Grey's anatomy). The facial nerve responsible for control of hearing, balance and transmission of images to the brain, smell, eye movement and other functions (Nanna et al., 2015). The facial nerve is embryologically derived and consequently innervates all structures originating from the second branchial
Any significant disturbance to the facial nerve may result in temporary facial weakness or paralysis, which in turn makes an incomplete eye closure inappropriate facial movement, dryness or excess fluid in the eye or mouth and alteration of taste (Jonghyuck et al., 2009).

The peripheral nerve injuries are still a common clinical problem. The recovery depends on the grade or the severity of the injury. As neurapraxia grade of injury requires remyelination of axon, axonotemetic grade of injury requires not only remyelination but also axonal regeneration. Also in the neurotmesis grade of injury, regeneration doesn't occur without surgery. After nerve injury followed by wallerian degeneration, it was the inflammatory response of the nervous system to axonal injury. This is primarily attributable to the production of cytokines, the mediator molecules of inflammation. The wallerian degeneration is significant to future regeneration (Yuval et al., 2016).

Hyaluronic acid is made by fibroblast cells. Commercial sources may be from Cocks combs, Chicken cartilage or Microbia Vegetarian source may be made via microbial fermentation (Carmela et al., 2014). Hyaluronic acid is a type of sugar (polysaccharide) that is present in body tissues such as in skin and cartilage. The development of non-invasive, non-toxic, and non-pollutant methods for the treatment of different illnesses represents a constant concern of scientists from the medical field worldwide (Zaleski et al., 2006). This category fit the methods based on the use of laser systems.

Hyaluronic acid was abundantly present in almost all biological fluids and tissue. It is found in the extra cellular matrix (ECM) of soft connective tissue that H.A. is involved in tissue repair and regeneration (Manjoo et al., 2015). Hyaluronic acid performs several structural tasks in the extracellular matrix (ECM) as it binds with cells and other biological components through specific and non-specific interactions. Several extracellular matrix proteins are stabilized upon binding to HA, which can be an ideal material for tissue engineering purposes (Anna et al., 2015). The aim of the present study was focused on the effect of Hyaluronic acid on facial regeneration and the regeneration time of facial nerve.

2. Materials and Methods

To conduct this study, a total of 8 adult bitches aged 1 to 1.5 year, weighing 18 -21 kg are used in this study. Dogs are accommodated in the same laboratory conditions by keeping them in special cages (1 dog per cage). The dogs were kept in cages 15 days before the operation of acclimatization.

The dogs were randomly divided into two equal groups as the followings each includes 4 dogs in group: In control group, the left facial nerve are transected and immediately sutured. Then, the clinical signs are recording till the end of the experiment. The macroscopic findings, EMG and neurohistopathological examination were done at the 12th week, post operation. Hyaluronic acid group similar procedure as in Control Group, but it was injected with Hyaluronic acid 0.01 mg/10 kg b.w in a single dose after 48 hrs of surgical operation. The macroscopic findings, EMG and neurohistopathological examinations were done at the 12 week, post operation similar as Control Group.

The dogs were fasted 24 hrs and water with draw for 12 hrs before the operation. The site of the operation was clipped and shaved carefully before the operation. The dogs were given a mixture of ketamine hydrochlorid 15 mg/kg b.w and xylazine hydrochlorid 5 mg/kg b.w. intramuscularly. The left cheek of face was prepare under aseptic technique. The operation was carried out by incision skin from end external ear 2 cm to cheek parallel with the maxilla, length of skin incision 3 cm. After opening the skin of the facial nerve cut off by using a surgical scalpel. Then, the two ends of nerve suture immediately with end to ends, anastomosis using of two sutures by 0.5 nylon are placed equidistantly around the nerve in the epineurium after that the skin closed with 0.3 silk suture. Dogs are given systemic antibiotic 10.000 IU procaine Penicillin, intra muscular injection daily for 3 days, post operation.
Clinical observations were followed up for all animals during 12 weeks according to macroscopic finding study in one period. The clinical finding includes the onset and the inability to eat food falling from the side of the mouth and messy eating, inability to close the eye, facial asymmetry, lip drooping, decrease response eyelid reflex, discharge of pus from the affected eye and excessive drooling.

Electrophysiological examination is a fairly non-invasive method of assessing functional neuromuscular disorders (Banu et al., 2013). The examination were carried out at 12 weeks post operation four dogs in each group, the facial nerve was separated 2 cm and isolated from the two sides of face (left and right) of the body, soaked in AD instrument chamber (filled with buffer solution) attached to negative, positive (recording) electrode clamps and stimulus electrodes. The conductive velocity was recorded from the multiplication of distance between recording electrode (mm) by time interval between capacity proximal and capacity distal (ms). The stimulations were performed at a square wave of 0.2 millisecond (ms) duration with a frequency of two pulses per second.

Microscopic examination of facial nerve sections was used to determine the number of schwann cells, arrangement of nerve fibers, the composition of new blood vessels formation, proliferation collagen fiber, neurocyte fibers and ranvier portion. The neurohistopathological examination was done with routine stain (Hematoxylin and Eosin stain). The left facial nerves were isolated from each dog and the nerve samples were using stay suture to keep the nerve tissues straight. After 12 weeks, the following macroscopic observation was obtained (degree of nerve coaptation, presence of adhesions and presence of thickening). For histopathological examination, a specimen from the nerve at the site of the anastomosis was taken to the repair of epineurial and axonal regeneration with less scarring less injury to the nerve blood supply (Adrian et al., 2016).

3. Results

In control group, the clinical signs after recovery from anesthesia, paralysis of the facial nerve appeared after 48 hrs after the operation (loss of sensory and motor function) with pain at the operation site as well as dogs inability to eat, usually fall of the food from the mouth and it lasted for several weeks on this case in the control group dogs as well as eating messy. Lifting food around the mouth, food falling from the side of the mouth, a runny excessive salivation, the inability to close the eye, rubbing and discharge from the eye. Inability to close eyelids, a large separation between the upper and lower eyelids, reduced or absent risk response and reflex eyelid, lack of facial symmetry, ear and lip drooping, collapse of the nose , patients deviation toward the side of the injured - Chronic (This can be seen occasional facial spasms) ,discharge of pus from the affected eye, Drowsiness or stupor. At week 4, the emergence of swelling in the face was abnormal and lasted for 3 weeks and then swelling disappeared of the end of week 12. It was not that the dog retrieves the reconsider entire health macroscopic. Hyaluronic acid Group: In Hyaluronic acid, paralysis of left side of facial nerve was clear post operation and all signs absent about eight days and on 10th days the dog returned to normal position but in laser group appears all signs and disappear on 14 days.

Conductive at 12 Week was post - operation

The facial nerve was isolated from right and left side of face via to electrophysiological examination at 12 week post operation. The results showed significant differences between control and hyaluronic acid, at P^ 0.05. However, significant differences were observed in the same group between left facial nerve and right facial nerve post operation as presented in Table - 1. The best conductivity was in
Hyaluronic acid Group (20.8 m/s) compared with control group (1.7 m/s) (Fig - 1 & 2).

**Histopathological Examination in Control group**

The histopathological examination of the longitudinal section of the facial nerve sections showed the several vacuolated, inflammation cells. Odema and swelling the neurocyte for 12 week shows the nervous tissues are between muscles fibers and adipose tissue the nervous tissue are present with subperinum as in Fig - 3. Moderate vacuolated degeneration which seen as size increase that to keep swelling of neurocyte as in Fig - 4. Besides, it shows decline of the vacuolated of neurocyte with waller in degeneration of Schwann cell which present in other filed precellular odema as in Fig – 5.

**Hyaluronic acid Group**

The histopathological examination of the longitudinal section of the facial nerve section Hyaluronic acid shows proliferation of collagen fiber of adipocyte and blood vessels for 12 week show there was mild edematous fluid with present collagen fibers subperineurium and between nervous fibers as in Fig - 6. Mild vacuolated and there was collagen between fibers present in some area with new blood vessels and degeneration of Schwann cell as in Fig - 7. Bindles of collagen fibers which present between fibers with surrounded the fibers and new blood vessels as in Fig - 8.

**Table - 1: Statistical analysis of Conductive Velocity of Control, Hyaluronic and Laser Croup at 12 week RFN: Right facial nerve. LFN: Left Facial Nerve**

<table>
<thead>
<tr>
<th>Group</th>
<th>At 16th PO Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>FN (OP)</td>
</tr>
<tr>
<td></td>
<td>1.7 ± 1.37*</td>
</tr>
<tr>
<td></td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>39.97 ± 1.78</td>
</tr>
<tr>
<td>H.A</td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>25.47 ± 2.61*</td>
</tr>
<tr>
<td></td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>34.12 ± 3.56</td>
</tr>
</tbody>
</table>

**Figure - 1:** Shows the facial nerves waves (A) left (B) right, and conductive velocity for control group at 12 weeks PO, the C.V measured by multiplicaled the distance between recording electrodes on time interval.
Figure - 2: Shows the facial nerves waves (A) left (B) right, and conductive velocity for H.A group at 16 weeks PO, the C.V measured by multiplied the distance between recording electrodes on time interval

Figure - 3: Section of facial nerve of control dog showing hemorrhage area in the tissue

Figure - 4: Section of facial nerve of control dog showing odematous as space between fibers

Figure – 5: Section of facial nerve of control dog showing swelling of neurocyte

Figure – 6: Section of facial nerve of H.A dog showing increase of Adipocytes proliferation of collagen fibers

Figure – 7: Section of facial nerve of H.A dog showing new blood vessels
4. Discussion

Our other results in the facial paralysis are loss of sensory function, pain, difficult eating and drinking. There are in control group with lifting food around the mouth, excessive salivation, not close the eye, rubbing absent risk response and reflex that continues for the 4 week. In Hyaluronic acid, paralysis of left side of facial nerve is clear post operation and all signs absent about 8 th day and on 10 th day and the dog returned to normal position. Biotechnology has provided tools and techniques to generate treatment for previously disease with low level laser therapy used in wound healing and nerve regeneration to improve the time regeneration that was agreed with Ahmad (2016).

The physical properties of H.A are important but there is an evidence to suggest that H.A may provide physiological, physicochemical and pharmacological advantage, H.A acts highly hydrophilic, it is a polymer that was well suited to applications requiring minimal cellular adhesion that was agreed with (Necas et al., 2008). The facial nerve paralysis appears moderate coaptation of the proximal and distal end of nerve stump, and show sever adhesions were observed. That in Control group, a good coaptation in the operated site. But in Hyaluronic acid group, a good coaptation with no complications (Necas et al., 2008).

The H.A is a naturally occurring linear polyelectrolyte that was found in biology. The functional groups available for cross linking are the hydroxyl and carboxyl groups. The hydroxyl groups may be cross linked via an ether linkage and carboxyl groups via an ester linkage. The facial nerve paralysis in the evaluation appears the significant differences that were observed in the different groups and were recorded in electrophysiological examination. The best group was Hyaluronic acid and Control.

The values of conduction velocity of Control and Hyaluronic acid one about the 1.7 m/s, 20.8 m/s, 1.7 m/s respectively. That means highly significant differences were observed among the groups. Due to treatment with Hyaluronic acid enhance the growth factor that was agreed with Zor et al. (2014). The hydrogel of H.A act as biomimetic - scaffold for peptides. The laser activated axon regeneration starts a few hours after the injury that agree with my study according to the absence of any effect of the laser on nerve regeneration (Ciro Ferreira et al., 2003). The high viscosity of H.A preparation appeared to retard in the growth of axon and non neuronal cells. The H.A normal component of intact and regeneration peripheral nerve is a fibroblast derived glycosaminoglycan which was believed to play an important that agree with Tona et al. (1999). The EMG findings, the occurrence of fibrillation potential positive sharp waves and complex repetitive discharges which has been also described in dog through Dokuzeylul et al. (2013).

In Control group, the several vacuolated, inflammation cells. Odema and swelling the neurocyte are the shown Hyaluronic acid shows proliferation of collagen fiber of adipocyte and blood vessels. But, the laser group showed congestion of blood vessels and degenerated of neurocyte fibers tissue. Engineering techniques may be offer a potential solution to clinical problems. Because of Hyaluronic acid its physicochemical properties, polyanimic polymer was versatile. The naturally occurring material was unfortunately that are exploited for chemical modification for use in the preparation. Single oxygen is an oxygen form the electrons of which are excited to a higher energy level in comparison that is agreed with Kogan et al. (2007).

Our results showed that the H.A of nerve regeneration was the best due to its mechanism of the cleavage of the glycosidic bonds between B - 4 - D glucuronic acid and B
- 3N – acetyl – D -glucosamine repeat units. It is interesting to consider that all efforts to determine the content and size of H.A in biological tissue and fluids have made the high assumption that chemical structure in the present study. We have also observed that the surgical sites treated with H.A appear more intense inflammation response compared with laser and control groups that depend on the immigration neurocytes and deposition of cell to enhance regeneration. The H.A called viso supplementation therapy with Hyaluronic acid that was agreed with Eleni et al. (2012). The Hyaluronic acid is very effective thought the studies by Sluka et al. (2003). That is play in nerve regeneration and agree with our study. The enhancing nerve regeneration of nerve injury was problem due to the effect on function nerve and to improved nerve regeneration using laser and to outcome of clinical microsurgery in the treatment of nerve injuries have a great relevance, iaues neurobiological alteration in neurons and Schwann cells that agree with the result of our study was consistent with other reports investigating the effect of laser He – Ne at 632 nm to nerve regeneration that were able to demonstrate stimulation of facial nerve in dogs model accelerated both axonal regeneration and the development of pereferential motor reinnervation that was agreed with Zor et al. (2014).

6. References

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