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Original Article

Histological assessment of potential inferior alveolar nerve injury following osteotomy of the mandibular buccal cortex using a piezoelectric saw

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Abstract

The present study aimed to evaluate the effect of Piezosurgery on histopathologic features of the inferior alveolar nerve (IAN) damage after osteotomy of the buccal cortex of the mandible using piezoelectric devices in Hamdani sheep. A total of ten healthy mature female sheep were included. Each side of the mandible underwent two different experiments: the first experiment operated directly on the mental nerve by touching and activating the piezo tip on the nerve for ten seconds for the left side and thirty seconds for the right side. In the second experiment, the inferior alveolar nerve was touched by an activated piezo tip inside the mandibular canal for ten seconds on the left side and thirty seconds on the right side. All the nerve samples underwent histopathological evaluation, and the scoring system was performed to assess the nerve structures. Mental nerves exposed to piezo tip for 10 seconds showed mild abnormality including disruption of the perineurium with the endoneurium remaining intact. Mental nerves exposed for 30 seconds showed moderate injury with destruction of the perineurium and moderate degeneration of nerve fibers, nevertheless, the endoneurium remained continuous with normal node of Ranvier. Severe damage of the inferior alveolar nerve was seen after exposure to piezo tip for 10 seconds, which showed sloughing of the perineurium and severe vacuolar degeneration of nerve fibers, partial disruption of the endoneurium; however, the axons were still intact. Inferior alveolar nerves exposed for 30 seconds revealed destruction of the perineurium, marked vacuolar degeneration of nerve fibers, focal damage of axon and loss of endoneurium (axonotmesis). Piezosurgery devices have the potential to cause severe nerve damage during surgery and should be used very carefully.

Keywords: Inferior alveolar nerve, Mental nerve, Nerve damage, Piezo surgery, Sheep

1. Introduction

The inferior alveolar nerve (IAN) is at risk of damage during various surgical procedures such as mandibular osteotomies for the correction of dentofacial deformities, nerve lateralization for the facilitation of implant placement, graft harvest from the ramus or chin area, and distraction osteogenesis [1]. Bilateral sagittal split osteotomy (BSSO) is the most common mandibular corrective operation for skeletal malocclusion, during which there is a risk of postoperative neurosensory deficit of the IAN [2]. Injury to the IAN may occur during various stages of surgery, including soft tissue dissection, osteotomy, or bone removal with cutting instruments [3].

In mandibular surgery, osteotomy and bone removal are routinely performed with the help of rotating instruments or oscillating saws. These traditional tools are highly effective in cutting bone tissue but are not selective for bone. Using these devices speeds up the surgical process, but it poses the risk of damaging the neurovascular bundle that runs within the bone [4].

Only a few evidence-based studies with different results have evaluated traumatic nerve injury after maxillofacial surgery using piezoelectric devices [5]. The significant advantage of piezosurgery is its selective cutting of mineralized bone. Frequencies higher than 50 kHz are needed to cut soft tissues. The piezoelectric device is designed to produce ultrasonic micro-vibrations of 60-210µm at a frequency of 25-30 kHz [6].

Piezoelectric surgery uses ultrasonic vibration for osteotomy and selectively removes the bone, which allows operations to be done with the minimum involvement of the soft tissues, such as blood vessels and nerves [7]. However, the previous literature has yet to report any evidence of scientific experiments to examine the possible nerve injury by the piezoelectric device [8].

The major disadvantage of piezosurgery is that it requires a short learning curve to attain maximum efficacy, which could be frustrating to the operator [9]. The concept

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of digital pressure the surgeon applies during cutting by traditional instruments is significantly different from that of piezoelectric surgery. The latter do not need pressure for cutting efficiency. In case more digital pressure is applied, it may lead to a decrease in micro-vibrations of the piezo tip or even stop it at all. When the micro-vibrations are prevented due to excessive pressure on the working tip, energy not used for cutting will be transformed into heat, which, if it continues enough, may damage the tissue [10].

The main goal of this experiment was to evaluate the structural abnormalities of the inferior alveolar nerve after osteotomy of the buccal cortex of the sheep's mandible.

2. Materials and methods

This interventional histological study was conducted on sheep at the research center of the University of Sulaimani from January 2023 to September 2023.

This research protocol was reviewed and approved by the Scientific Ethics Committee of the Kurdistan Higher Council for Medical Specialties (approval number 1995, date 2/11/2022).

The sample of this study was ten healthy, two-year-old, mature female Hamdani Sheep, weighing 50-70 kg, which were obtained from the Qaragol slaughterhouse of the Sulaimani Province/Iraq. Immediately after the decapitation of the animal, the mandible was cut from the head and split at the midline into two halves, right and left. The mandible of sheep underwent an experiment within 3 hours of the slaughtering. The surgical field was prepared and draped. Using scalpel No.15, the mucoperiosteal flap is harvested from the bone, taking care not to cut the nerve at the mental foramen. For osteotomy, the piezo device (NSK VarioSurg3 piezo Ultrasonic devices) was set on its highest setting for all cuts. A jet of the physiological saline solution was on maximum provided constant cooling. For cutting reason (tip SG1) was used. A nerve probe was used to carefully raise the nerve from the canal.

Two experiments were done on each side of the mandible: On one side, direct application was used on the nerve that comes out of the mental foramen, and the second inside the inferior alveolar canal.

For the first one, a pen was used to mark the nerve 1cm away from the mental foramen. The activated Piezo tip was applied to the nerve by touching it with determined pressure and for a determined duration.

For the second experiment, the first careful decortication and canal unroofing was carried out at the anterior part of the mandible using applied protocols of IAN lateralization or distalisation on humans. The IAN was protected by a thin bent spatula inserted in the mental foramen. When the nerve became visible to the naked eye, the activated piezo tip was applied to the IAN at one cm posterior from the mental foramen. A total of 40 samples were used in this study: twenty pieces of the nerve treated outside of the bony canal and 20 inside the bony canal. The nerve segments were cut, labeled, and allocated into four groups as follows:

Group 1: n=10, treated or exposed at the mental foramen where the nerve comes out and exposed to activated piezo tip for 10 seconds for the left side.

Group 2: n=10, treated or exposed at the mental foramen where the nerve comes out and exposed to activated piezo tip for 30 seconds for the right side.

Group 3: n=10, treated or exposed the nerve inside the bone in the mandibular canal just 1cm posterior to the mental foramen exposed to activated piezo tip for 10 seconds for the left side.

Group 4: n=10, treated or exposed the nerve inside the bone in the mandibular canal just 1cm posterior to the mental foramen exposed to activated piezo tip for 30 seconds for the right side.

2.1. Histopathological Examinations

All the nerve samples were sliced and fixed for 24 hours in a 10% paraformaldehyde solution in 0.1 M phosphate buffer (PB, pH 7.0-7.5) before being rinsed in PB. Serial sections of 4μm thickness were produced after the tissues were soaked in paraffin wax and stained with H&E. The slides were inspected using an eyepiece grid under a microscope at 20-400 magnification by two pathologists blind to the study. The samples were evaluated manually with a conventional light microscope (Leica, Germany) via computer-assisted image analysis software to examine slices (Am ScopeTM, Japan). In the present investigation, IAN damage was scored using the grading system proposed by Schaeren et al. [11], which was modified by the authors of the present study to the following scoring system: Grade 0, intact connective tissue organization with no nerve damage; Grade 1= damage of perineurium with intact nerve fiber; Grade 2= 20-25% of axonal degeneration; Grade 3,= 26-50% of axonal degenerations; Grade 4= ≥75% of axonal degeneration; Grade 5= complete disruption of the axon (axonotmesis) and also partial injury to the endoneurium.

2.2. Statistical Analysis

Statistical analysis was performed using SPSS statistics version 24 (IBM, SPSS Inc, Chicago, USA). A P-value < 0.05 was considered statistically significant. Data were analyzed by two-way ANOVA evaluating the effect of the piezo tip in/on both the canals or the bone, respectively.

3. Results

The microscopic longitudinal section of nerve fiber showed various histopathologic features, for example the group one, in which at the mental foramen where the nerve comes out and exposed to activated piezo tip for 10 second showed mild abnormality including disruption of perineurium that cover the bundle of nerve fibers, while the endoneurium that surrounded the individual nerve fiber or axon still intact, each nerve fiber showed normal histologic structure (Fig. 1) recorded by Grade 1 vs. to the second group at the mental foramen where the nerve comes out and exposed to activated piezo tip for 30 seconds showed Grade 3 and had moderate injury as in (Fig. 2) displayed destruction of perineurium and unorganized arrangement in nerve fiber (Fig. 2b), moderate degeneration of nerve fiber that described by clear vacuole with intact centrally located Schwan cells, also the endoneurium remain continuous with normal morphologic feature for node of Ranvier (Fig. 2c,d), while severe lesion and high grade seen in third groups in which the nerve inside the bone in the mandibular canal just 1cm posterior to mental foramen exposed to activated piezo tip for 10 seconds showed damage or sloughing of perineurium completely with severe vacuolar degeneration of nerve fiber (Fig. 3a-d), also partially disruption of endoneurium buts the axon still remain intact histologically and recorded as Grade 4, (Fig. 4c,d)

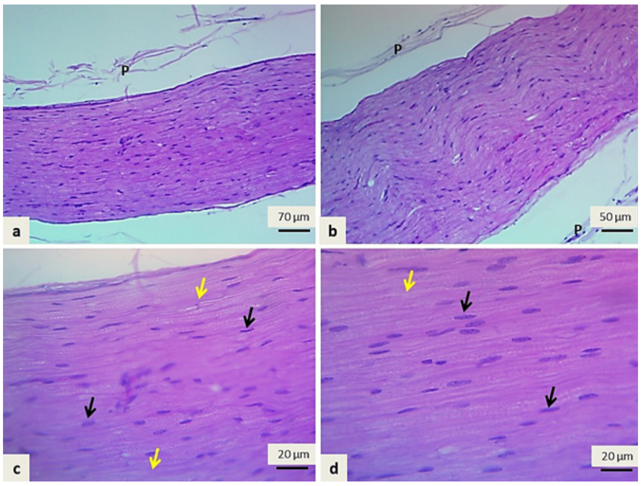

Fig. 1. Light microscopic longitudinal section of nerve fiber in sheep of the G1 revealed Grade 1; a and b: Sloughing of perineurium (P) with well-organized nerve fibers, c, and d: Normal architectures of individual nerve fiber that surrounded by intact endoneurium with normal features of axon and node of Ranvier (yellow arrows) with Schwan cells as indicated by black arrows, (H&E stain).

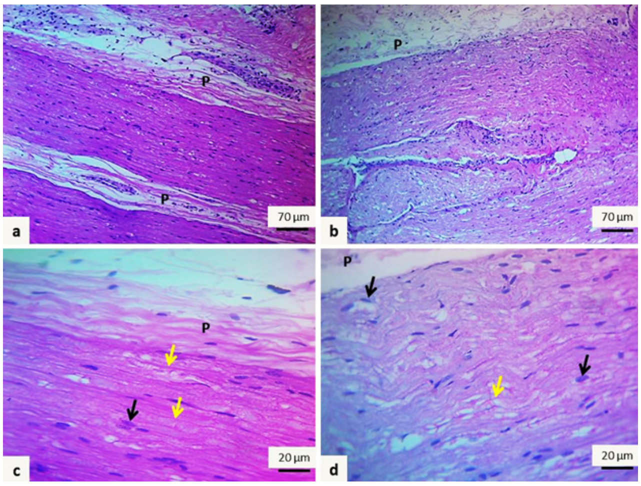

Fig. 2. Light microscopic longitudinal section of nerve fiber in sheep of the G2 revealed Grade 3; a and b: Disruption of perineurium (P) and disorganized nerve fiber in section (b), c and d: Moderate degeneration of nerve fiber that characterized by clear vacuole with normal features of Schwan cells (black arrows) surrounded by intact endoneurium with normal morphology node of Ranvier and as indicated by yellow arrows, (H&E stain).

in comparison to the fourth groups that exposed for 30 seconds that revealed completely destruction of perineurium, marked vacuolar degeneration of nerve fiber (Fig. 4a-d) with focal damage of axon and loss of endoneurium (axonotmesis) marked as Grade 5, (Fig. 4c,d).

Table 1 described the statistical analysis of histopathologic abnormalities that were detected in each group and showed the nerve degeneration in G1 had a significant minimum degree (38.83±2.24) vs. the other groups (P=0.000), while the G4 recorded the highest level (87.50±2.40) in nerve abnormality significantly (P=0.001), whereas G3 showed non-significant (P=0.07) decreasing in nerve degeneration vs. to the G4.

Regarding the epineurium and endoneurium disturbance, the minimum damage (3.83±0.5 and 01.33±0.49)

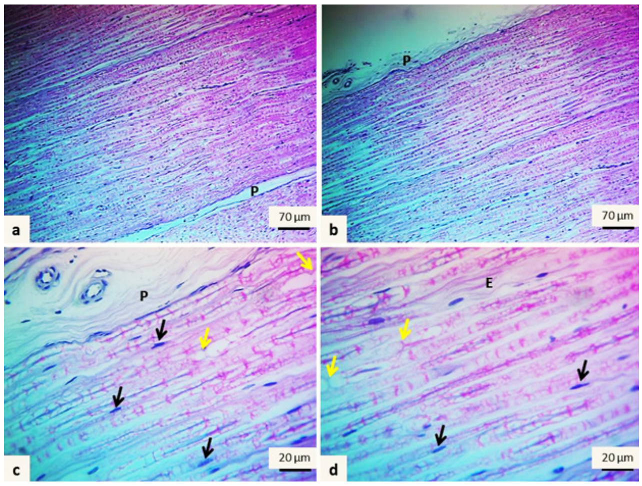

Fig. 3. Light microscopic longitudinal section of nerve fiber in sheep of the G3 that revealed Grade 4; a and b: Damage of perineurium completely (P), b-d: Marked vacuolar degeneration of nerve fiber with normal histological features of Schwan cells (black arrows) and node of Ranvier (yellow arrows), partial damage of endoneurium (E) and intact axon, (H&E stain).

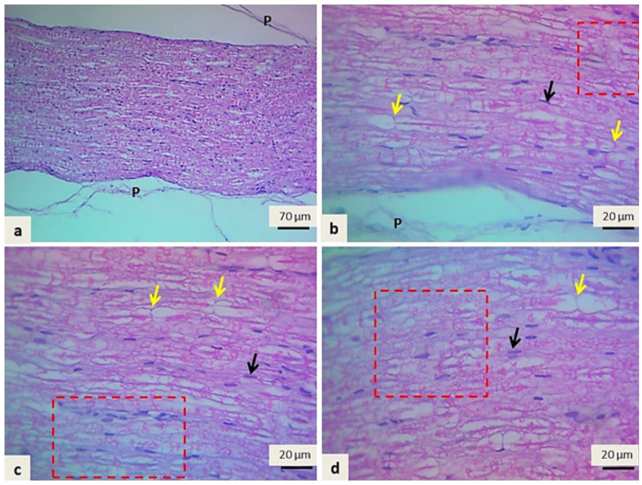

Fig. 4. Light microscopic longitudinal section of nerve fiber in sheep of the G4 revealed Grade 5; a and b: Disruption of perineurium completely (P), b-d: Severe degeneration or vacuolization of the nerve fiber with intact Schwan cells (black arrows) and node of Ranvier (yellow arrows), focal damage or loss of endoneurium and axon as indicated by red dash line, (H&E stain).

Table 1. The average score of IAN and mental nerve damage of the four groups.

Lesions	G1	G2	G3	G4
Nerve degeneration	38.83±2.24***	64.83±2.89**	80.00±4.46	87.50±2.40***
Epineurium damage	3.83±0.5***	59.16±7.89**	90.83±1.40	97.00±1.63***
Endoneurium damage	01.33±0.49***	8.10±1.77**	69.66±6.61	81.33±4.25***
Axonal damage	01.00±0.51***	1.83±0.94**	7.83±0.94***	74.16±4.36***

Within each row, values expressed by Mean ±SE, the symbol *** regarded as P ≤0.05 considered significant.

respectively found in the G1 in comparison to the other groups by a significant degree (P=0.001 and P=0.000), whereas, the maximum damage was found in the G4 significantly (97.00±1.63 and 81.33±4.25) correspondingly, while, in G3 the sloughing of the epineurium and endoneurium less than G4 by non-significant value (P=0.07 and P=0.12).

The axonal damage showed variable value; in G1 significant (P=0.00), no damage was observed vs. to the G3 and G4 that arranged from mild-severe damage as in G4, even the significant value (P=0.00) seen in comparing G3 to G4 ($P \leq 0.05$), while non-significant relation (P=0.17) was found between G1 and G2.

4. Discussion

Postoperative pain, facial swelling, and trismus are stressful conditions that are faced after impacted mandibular molar surgery. Thus, oral surgeons try to decrease postoperative complications via different approaches, including piezosurgery [12]. Piezosurgery is a novel technology that uses piezoelectric vibration in the application of cutting bone tissue [13]. The manufacturer claims that bone tissue may be cut selectively without injurious effects on all soft tissues, including nerves, blood vessels, Schniederian membrane, and Dura matter [14].

In the current study, injury to IAN by Piezo tip has been assured in both methods: inside and outside the bony canal. In this way, the study raises the question of the safety of soft tissue during piezosurgery. The mechanism of injury is not clear yet, but direct trauma by traction, crushing, heat, and possibly vibration are among the suggested causes. Disagreement with the previous studies, which examined the neurosensory impairments following oral and maxillofacial surgery by using piezoelectric devices with varying outcomes. All of them mentioned no neural deficit [15, 16].

The amount of injury depends on the power applied; in the current study, the same power was used for different periods and gave two different parameters of injury. Hence, the theory of traction force may lose its applicability in the current situation [17]. Another fact that does not support the traction injury theory is the presence of injury not only in the first group, where the nerve was out of the canal but even inside the bony canal, there is injury, which is much more severe than in the first group.

In our data, by histopathologic utilization, the amount of injury in the G1 and G2 was less compared to the G3 and G4. This finding can be explained by, first, the presence of flexibility of the nerve tissue that gives some absorption effect; second, the absence of hard ground (bony tissue) under the nerve that prevents crushing injury; and third, the lack of cutting that minimizes the heat generation [18]. Another possible factor of damage is the heat generated during piezosurgery. The amount of heat generated is directly proportional to the amount of force applied. The application of pressure can be considered habitual for surgeons who use rotary instruments for bone cutting [19]. We can expect some heat generation in the third and fourth groups where the nerve is in the bony canal, but the theory is not applicable in the first and second groups where the nerve is out of the bony canal. Also, the other hypothesis is that the IAN has a fixed location inside the mandibular canal and may undergo minimum or no movement when contacted by the piezoelectric tip. The nerve is vulnerable to traumatic injury in groups 3 and 4, where the nerve was severely injured. The longer an active piezoelectric tip remains on a nerve, the more damage it does; hence, time is a crucial component in nerve injury. Accidental slippage of the triggered piezo tip might result in direct mechanical nerve structure damage.

This study focused on the duration of the application of the piezo tip exposed to the tissues. The histological findings confirmed the effect of duration on the amount of injury to be expected. The amount of injury in group 4 was the maximum that showed axonal damage vs. corresponding groups. Any surgery around IAN takes much more than 30 seconds (the maximum duration used in the experiment); it may take 10 to 30 minutes, so we can imagine how much damage the nerve may have during such procedures [20]. Similar to the previous studies, the current results showed the disruption or sloughing in the epineurium in most groups. This is a minimum level of injury due to piezo application. According to Pencek et al. [21], the disruption of perineurium alone is enough to make the conduction velocity to be lower by 30-40%. Based on Sunderland’s classification of peripheral nerve injury, even the first degree of injury clinically may lead to functional loss at least for some time [22]. While the following studies did go along with our results, Spinelli et al. found that piezosurgical tools result in less blood loss and a lower incidence of postoperative hematoma, edema, and nerve damage following bimaxillary orthognathic surgery than standard saws [23]. The study done by Brockmeyer et al. exhibited a reduction in somatosensory impairment and a faster recovery of somatosensory function following orthognathic surgery of the mandible utilizing piezosurgery as compared to conventional saws [24]. Also, in disagreement with our data, the former studies proved that the sequelae following the removal of third molars impacted the jaw using piezo tomes or a standard handpiece. Comparing these trials found that the piezosurgery group experienced reduced postoperative pain and a higher quality of life assessment. Still, there were no changes in the incidence of paraesthesia or changed nerve sensitivity across groups [25-27].

The different results of previous studies evaluating neurosensory impairment after osteotomy with piezosurgery may be due to several factors, including the diversity of surgical procedures with the variety of bone-cutting instruments and the use of surgical procedures such as BSSO and impacted tooth removal that have confounding effects. The danger of neurosensory impairment following BSSO is intrinsically high, and it may conceal the underlying relationship between nerve injury and the bone-cutting mechanism [28]. On the other hand, the risk of IAN injury following impacted tooth extraction is low, and a large sample size is required to detect the actual difference in the rate of neurosensory disturbance between the two types of bone-cutting systems [24]. The other factor is the subjective assessment of the neurosensory disturbances, which can be influenced by the patient's experiences, prejudices, and memories. It has been claimed that somatosensory perception is a complicated system comprised of several distinct sensory properties, none of which can be assessed by most clinically utilized sensitivity tests [29].

Postoperative pain, facial swelling, and trismus are stressful conditions that are faced after impacted mandibular molar surgery. Thus, oral surgeons try to decrease postoperative complications via different approaches including piezosurgery [12]. Piezosurgery is a novel technology that uses piezoelectric vibration in the application of cutting bone tissue [13]. The manufacturer claims that bone tissue may be cut selectively without injurious effects on nearby all soft tissues including nerves, blood vessels, Schniederian membrane, and Dura matter [14].

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The fact that the amount of injury depends on the power applied, in the current study the same power applied for different periods and gave two different parameters of injury. Hence the theory of traction force may lose its applicability in the current situation [17]. Another fact that does not support the traction injury theory is the presence of injury not only in the first group where the nerve was out of the canal but even inside the bony canal there is injury and it is much more serious than in the first group.

In our data, by histopathologic utilization the amount of injury in the G1 and G2 was less compared to the G3 and G4. This finding can be explained by first, the presence of flexibility of the nerve tissue that gives some absorption effect, second, the absence of hard ground (bony tissue) under the nerve that prevents crushing injury, and third, the absence of cutting that minimizes the heat generation [18]. Another possible factor of injury is the heat generated during piezosurgery. The amount of heat generated is directly proportional to the amount of force applied. The application of pressure can be considered habitual for surgeons who use rotary instruments for bone cutting [19]. We can expect some heat generation in the third and fourth groups where the nerve is in the bony canal, but the theory is not applicable in the first and second groups where the nerve is out of the bony canal. Also, the other hypothesis is that the IAN has a fixed location inside the mandibular canal and may undergo minimum or no movement when contacted by the piezoelectric tip, the nerve is vulnerable to traumatic injury in groups 3 and 4, where the nerve was severely injured. The longer an active piezoelectric tip remains on a nerve, the more damage it does; hence, time is a crucial component in nerve injury. Accidental slippage of the triggered piezo tip might result in direct mechanical nerve structure damage.

This study focused on the duration of the application of the piezo tip exposed to the tissues. The histological findings confirmed the effect of duration on the amount of injury to be expected. Amount of injury in the group 4 is maximum that showed axonal damage vs. corresponding groups. Any surgery around IAN takes much more than 30 seconds (the maximum duration of time used in the experiment), it may take from 10 to 30 minutes, so we can imagine how much damage the nerve may have during such procedures [20]. Similar to the previous studies, the current results showed the disruption or sloughing in the epineurium in the majority of groups. This is a minimum level of injury due to piezo application. According to the work of Pencek et al. [21] the disruption of perineurium alone is enough to make the conduction velocity to be lower by 30-40%. Based on Sunderland's classification of peripheral nerve injury, even the first degree of injury clinically may lead to functional loss at least for some time [22]. While the following studies did go along with our results; Spinelli et al. found that piezosurgical tools result in less blood loss and a lower incidence of postoperative hematoma, edema, and nerve damage following bimaxillary orthognathic surgery than standard saws [23]. The study done by Brockmeyer et al. exhibited a reduction in somatosensory impairment and a faster recovery of somatosensory function following orthognathic surgery of the mandible utilizing piezosurgery as compared to conventional saws [24]. Also, in disagreement with our data, the former studies proved that the sequelae following removal of third molars impacted the jaw using either piezotomes or a standard handpiece comparing, these trials found that the piezosurgery group experienced reduced post-operative pain and a higher assessment of the quality of life, but there were no changes in the incidence of paraesthesia or changed nerve sensitivity across groups [25-27].

The different results of previous studies evaluating neurosensory impairment after osteotomy with piezosurgery may be due to a number of factors, including the diversity of surgical procedures with the diversity of bone cutting instruments, and the use of surgical procedures such as BSSO and impacted tooth removal that have confounding effects. The danger of neurosensory impairment following BSSO is intrinsically high, and it may conceal the underlying relationship between nerve injury and the bone-cutting mechanism [28]. On the other hand, the risk of IAN injury following impacted tooth extraction is low, and a large sample size is required to detect the actual difference in the rate of neurosensory disturbance between the two types of bone-cutting systems [24]. The other factor is the subjective assessment of the neurosensory disturbances, which can be influenced by the patient's experiences, prejudices, and memories. It has been claimed that somatosensory perception is a complicated system comprised of several distinct sensory properties, none of which can be assessed by the majority of clinically utilized sensitivity tests [29].

5. Conclusion

The present study showed that piezosurgery devices had the potential to cause severe damage to the inferior alveolar nerve and moderate damage to the mental nerve during surgery and should be used very carefully.

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Authors' contributions

SHQ and KAK developed the project idea. SHO performed the experiment and surgery. SMAH examined the data and double-checked the Histopathological results. The work has been read and approved by all authors.

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Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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