Anatomical Structures in the Rabbit Carpal Tunnel: Comparison with Human

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flexor retinaculum	Abstract: In this study, it was aimed to reveal the morphological and morphometric changes of anatomical structures passing through the carpal tunnel, which is an entrapment neuropathy location for the median nerve. It was also aimed to evaluate the potential of rabbits as an animal model for carpal tunnel research. New Zealand rabbit cadavers were enrolled, and their wrists were investigated using the histological method in this study. ImageJ was used to measure histological sections stained with hemoxylin-eosin. The carpal tunnel was examined at three levels: entrance, inside, and exit. Flexor retinaculum comprises two layers, encircling the carpal tunnel from the palmar side. The deep and superficial digital flexor tendons, and radial carpal flexor tendon were observed within the carpal tunnel but not the flexor pollicis longus tendon. The median and ulnar vascular nerve bundles reside between two layers of flexor retinaculum in the carpal tunnel. The areas of these anatomical structures were measured from images at three levels. Since the flexor retinaculum was located at the entrance and inside, the carpal tunnel area was measured at both levels.
Received: 18 October 2023 Accepted: 30 December 2023	The narrowing in the area from the carpal tunnel entrance to the inside of the carpal tunnel in rabbits and the decrease in connective tissue bring the anatomical structures here closer together. This clearly demonstrates that the carpal tunnel is an entrapment neuropathy area, particularly for the median nerve. Upon comparing rabbit and dog carpal tunnel anatomy, it has been shown that rabbits exhibit a greater resemblance to humans, particularly with regard to the flexor retinaculum. This study emphasizes the importance of using the rabbit model to gain insights into carpal tunnel syndrome. It demonstrates similarities between rabbit and human anatomy, underscoring the value of this animal model for future research.

Introduction

The carpal tunnel is an osteofibrous canal located along the palmar aspect of the wrist, formed the carpal bones and the flexor retinaculum. This tunnel contains flexor tendons, the median nerve, median artery, and median vein, which are surrounded by the flexor retinaculum from the palmar side (1-3). The carpal tunnel syndrome (CTS), which is a common entrapment neuropathy in humans and is caused by compression of the median nerve in the wrist region (4, 5). This condition can lead to loss of work and time, and treatment can be economically costly (6-9). Enhancing the diagnostic precision of carpal tunnel syndrome (CTS) might be achieved with a comprehensive analysis of the anatomical characteristics of the median nerve.

Apart from carpal tunnel syndrome in humans, entrapment neuropathy of the ulnar nerve is also mentioned in this region. The region where the ulnar nerve passes through the carpal canal is called the Guyon canal (10-14).

Understanding the normal morphology of the carpal region contributes to the diagnosis and treatment of carpal tunnel syndrome. The carpal tunnel investigations conducted on animal models has also enhanced comprehension of the carpal tunnel and carpal tunnel syndrome (CTS) (1, 15, 16). Rabbits have been commonly utilised as a preferred animal model in carpal tunnel investigations due to their resemblance to humans (1, 5, 15-18). Moreover, rabbits are particularly preferred for CTS study due to their convenient accessibility and cost-effective feeding and care conditions (17-20). Nevertheless, these investigations often contain insufficient and inaccurate anatomical information, and there is no detailed morphometric study on the anatomical structures passing through the carpal tunnel in rabbits.

The objective of this study was to assess the morphological and morphometric characteristics of the anatomical structures passing through the carpal tunnel and obtain accurate reference values in rabbits using histological sections.

Materials and methods

Animal material

In this study, cadavers of thirteen adult New Zealand rabbits (7 male and 6 female) (*Oryctolagus cuniculus L.*) were used, which prepared for a previous anatomical study. The mean weight of rabbits was determined to be 2113 ± 299.70 g, with a range of 1774–2660 g. These rabbits were anesthetized with an intramuscular mixture of xylazine (5 mg/kg, Rompun; Bayer) and 35 mg/kg ketamine (Ketanes; Alke) and weighted. Then, they were exsanguinated by make an incision in their hearts. They were fixed by administering 10% formaldehyde through the aorta with a plastic catheter. For around nine months, the cadavers were kept in a 10% formaldehyde solution. After, performing histological sections were prepared from the cadavers. The study was approved by Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (No: 645583101/2017/141).

Histological section (HS)

Carpal tissue was preserved in 10% buffered formalin (NBF) and decalcified in 10% nitric acid. It was trimmed after sufficient decalcification for around 5 days. After multiple treatments with xylol and increasing alcohol solutions (70°, 80°, 90°, 96°, and 100°), the tissues were taken to the Leica TP1020 tissue tracking device and blocked in paraffin. These blocks were cut with a microtome (Leica RM 2135) and stained with hematoxylin and eosin (H&E) (21). At each three levels of carpal tunnel, serial sections of 5 μ m thickness were taken at 5 x 5 μ m intervals. The digital images taken using an Olympus SZX-LGR66 stereomicroscope were stored. Histological slices were photographed with a stereomicroscope and measurement paper. After transferring the photos to BSD-2-licensed ImageJ, measurements were taken.

Measurements

The carpal tunnel was examined at three levels: entrance (1. Level), inside (2. Level), and exit (3. Level). The first level was defined as the entrance of the carpal tunnel at the ends of the radius and ulna, where the palmar projection of the accessory carpal bone could be seen. The second level was defined as the inside of the carpal tunnel and included the proximal row of the carpal bones. The third level was defined as the exit of the carpal tunnel, where the proximal row of carpal bones ends and the distal row of carpal bones begins (Figure 1).

The area of the deep digital flexor tendon, superficial digital flexor tendon, and radial carpal flexor tendon that are passing through the carpal tunnel were measured. These measurements were taken by including synovial sheaths. The area of the median and ulnar nerve bundles were measured. These measurements were taken by including the fibrous band surrounding these structures. The inside area



Figure 1: Images from histological section at three levels (H&E, x20 magnification). A: carpal tunnel entrance (1. Level), B: carpal tunnel inside (2. Level), C: carpal tunnel exit (3. Level)



Figure 2: Image from histological section at the carpal tunnel inside (2. Level) (H&E, x20 magnification). CR: radial carpal bone, CI: intermediate carpal bone, CU: ulnar carpal bone, CA: accessory carpal bone, a; deep digital flexor tendon, b; superficial digital flexor tendon c; radial carpal flexor tendon, d; abductor pollicis longus tendon, e; radial carpal extensor tendon, f; common digital extensor tendon, g; primum and secundum digital extensor tendon, j; dorsal ramus of ulnar nerve, ulnar artery and ulnar vein, k; ulnar nerve bundle (palmar ramus of ulnar nerve, arteriole and venule), I: radial artery, accessory cephalic vein and radial nerve, m; median nerve bundle (median nerve, median artery, caudal interosseus artery, median vein and caudal interosseus vein)

of the median artery was measured by aligning the epithelial layer was measured. The wall thickness, including the epithelial and muscular layers of the median artery, was measured. These measurements were taken from images at three levels. The lengths of both layers of the flexor retinaculum were measured. Furthermore, the carpal tunnel area was measured between the flexor retinaculum and the bones. Since the flexor retinaculum was located at the entrance and inside, the carpal tunnel area was measured at both levels. The connective tissue area was calculated by subtracting the area of these anatomical structures from the area of the carpal tunnel (Figure 2, 3).

Statistical evaluation

The statistical analysis of the data was performed using the SPSS Statistics 21.0 software. The tables present the data as Mean value ± Standard deviation (Mean±Std).

Results

The carpal tunnel was investigated on three different levels. These were the entrance of the carpal tunnel (1. Level), the inside of the carpal tunnel (2. Level), and the exit of the carpal tunnel (3. Level) (Figure 1).



Figure 3: Images from histological sections of tendons and vascular nerve bundles in the carpal tunnel (H&E, A, B, C, E, x40 magnification and scale 200 μ m, D, x100 magnification and scale 100 μ m, Olympus-BX51, Light microscope). A; deep digital flexor tendon (black star), superficial digital flexor tendons (black arrow), B; radial carpal flexor tendon (black star), C; median nerve bundle (black arrow), median artery (black star), median nerve (white star) D: ulnar nerve bundle (black arrow), E: superficial (black arrow) and deep (white arrow) layers of the flexor retinaculum, CA; accessory carpal bone

It was found that the deep digital flexor, the superficial digital flexor and the radial carpal flexor tendons were within the carpal tunnel. It was observed that the median nerve bundle included the median nerve, the median artery, the median vein, the caudal interosseus artery and the caudal interosseus vein. The palmar ramus of the ulnar nerve, arteriole, and venule were located in the ulnar nerve bundle. The flexor retinaculum was bilayered as superficial and deep. These bundles were located between these two layers within the carpal tunnel at both sides. In the carpal tunnel, the deep digital flexor tendon and the radial carpal flexor tendon were observed to be in a single piece, whereas the superficial digital flexor tendon was observed to be in three separate parts (Figure 2, 3).

It was found that the area of deep flexor tendons decreased from level 1 to level 3, while the area of the radial carpal flexor tendon increased. It was observed that the area of Table 1: The measurements of tendons in the carpal tunnel in histological sections (HS) at the three levels. PA; deep digital flexor tendon area, SA; superficial digital flexor tendon area, RA; radial carpal flexor tendon area

	1. Level		2. Level		3. Level	
	n	Mean±Std	n	Mean±Std	n	Mean±Std
PA(mm ²)	9	6.16±0.74	10	6.03±0.39	6	5.66±0.55
SA(mm²)	9	1.91±0.44	10	1.86±0.25	6	2.10±0.29
RA(mm²)	8	0.17±0.03	9	0.21±0.03	5	0.23±0.03

Table 2: Comparison of the measurements of vascular nerve bundles in the carpal tunnel in histological sections (HS) all levels. MNBA; median nerve bundle area, MAA; median artery area, MAWT; median artery wall thickness, UNBA; ulnar nerve bundle area

	1. Level		2. Level		3. Level	
	n	Mean±Std	n	Mean±Std	n	Mean±Std
MNBA(mm²)	8	0.91±0.32	10	0.97±0.28	7	0.75±0.29
MAA(mm ²)	7	0.17±0.02	10	0.19±0.09	4	0.14±0.03
MAWT(mm)	6	0.04±0.01	10	0.05±0.01	4	0.04±0.01
UNBA(mm ²)	7	0.26±0.04	10	0.30±0.04	6	0.35±0.04

Table 3: Comparison of the measurements in histological sections (HS)two levels. CTA; carpal tunnel area, SFRL; superficial flexor retinaculumlength, DFRL; deep flexor retinaculum length

	1.	1. Level		Level
	n	Mean±Std	n	Mean±Std
CTA (mm²)	10	18.34±1.14	10	17.50±0.49
SFRL (mm)	10	9.14±0.30	9	8.33±0.24
DFRL (mm)	10	6.37±0.34	9	5.92±0.19

Table 4: Percentage changes in the areas of the anatomical structures within the carpal tunnel and the proportional differences of these changes during the transition from level 1 to level 2. CTA; carpal tunnel area, PA; deep digital flexor tendon area, SA; superficial digital flexor tendons area, RA; radial carpal flexor tendon area, MNBA; median nerve bundle area, UNBA; ulnar nerve bundle area

	Area%	Ratio
CTA	4.58 🕕	
РА	2.11	0.87
SA	2,62 🞵	0.22
RA	23.53	0.27
MNBA	6.59	0.58
UNBA	15.38	0.29
Connective Tissue	8.96 🕕	2.23

superficial flexor tendons was decreased at the inside of the carpal tunnel (Table 1).

It was observed that the measurements of MNBA (median nerve bundle area), MAA (median artery area), and MAWT (median artery wall thickness) were increased at the inside of the carpal tunnel. It was found that the area of ulnar nerve bundle increased from level 1 to level 3 (Table 2).

Measurements of the flexor retinaculum and carpal tunnel were taken from the 1st and 2nd levels, where the carpal tunnel was formed. The lengths of both the superficial and profound layers of the flexor retinaculum were decreased in the transition from level 1 to level 2. A similar reduction in carpal tunnel area was also seen (Table 3).

While passing through from level 1 to level 2 in the carpal tunnel, the percentage changes in the areas of the anatomical structures and the proportional differences of these changes in the carpal tunnel are shown in Table 4. In both levels, the connective tissue was calculated by taking out these anatomic structures from the carpal tunnel area. Based on this data, while the percentage changes in areas of the radial carpal flexor tendon and both nerve bundles were increasing, other structures' were decreasing. However, in terms of proportionality (the ratio of these structures' areas to the carpal tunnel area), the areas of all tendons and both nerve bundles have shown an increase, whereas the amount of connective tissue has seen a reduction.

Discussion

The carpal tunnel is a groove between the carpal bones and the flexor retinaculum. In humans, the entrance to the carpal tunnel is formed by the proximal row of carpal bones, especially the accessory carpal bone. The exit is shaped by the distal carpal bone row and especially the hook of the hamate bone (22-24). Since the hamate bone has no hook in dogs, the carpal tunnel is formed only by the accessory carpal bone (25). Since the bony roof of the carpal tunnel in rabbits is similar to that in dogs, in this study the carpal tunnel was examined at two levels, as in dogs.

In the morphology of the carpal tunnel in rabbits, unlike humans, it has been determined that one deep digital flexor tendon instead of four, three superficial digital flexor tendons instead of four, and the flexor carpi radialis tendon instead of the flexor pollicis longus tendon pass through this tunnel. In addition, it has been determined that the median nerve bundle in rabbits, unlike humans, is located more superficially between the two layers of the flexor retinaculum. It has been observed that the ulnar nerve package is located between the two layers of the flexor retinaculum, which resembles the Guyon canal in humans (23, 26). It has been reported that in dogs, unlike rabbits, the flexor retinaculum consists of two layers, and the superficial digital flexor tendon is located between these two layers. Additionally, it was observed that the ulnar and median nerve packages were located under the deep layer of the flexor retinaculum differently in rabbits (25). It should be emphasized that there is radial carpal flexor tendon but no flexor pollicis longus tendon in the carpal tunnel in rabbits do not agree with the previous studies (1, 2, 27).

The carpal tunnel area of rabbits was noticed to be approximately 1/9 that of humans (4, 6, 28). In dogs, taking the boundary of the deep layer of the flexor retinaculum, the carpal tunnel area of rabbits was seen to be approximately 1/7 that of dogs, and the superficial layer boundary of it was 1/13 that of dogs (25).

In humans, the ratio of the area of the tendons passing through the carpal tunnel to the carpal tunnel area has been reported to be approximately 58–59% (28). This rate was found to be slightly low and 46 % in this study conducted on rabbits at carpal tunnel inlet. It is thought that the discrepancies in these rates may be due to different tendons and numbers of tendons passing through the carpal tunnel.

The morphological profile of the median nerve (cross-sectional area, flattening ratio and circularity) has been proven to be the most effective parameter for evaluating compression neuropathy other than nerve conduction tests. Additionally, the assessment of flexor retinaculum bowing is also regarded as a significant criterion in the diagnosis of carpal tunnel syndrome (CTS) (29-31). In the determination of the flattening ratio of the median nerve in the human, the long and short diameters of the nerve bundle are used. In humans, the median nerve normally presents in the carpal tunnel as a whole, with all nerve fibers surrounded by a sheath. However, in rabbits, the median nerve fibers are dispersed, and present as a vascular nerve bundle together with the median artery and median vein. Therefore, it is thought that it is more appropriate to measure the area of the vascular nerve bundle in rabbits instead of the flatting ratio.

It has been reported that the area of the median nerve is an average of 8 mm² (7.16-8.7 mm²) in the carpal tunnel in humans by ultrasonography (29-35). When calculating the ratio of the median nerve area to the carpal tunnel area, it is found to be approximately 5% in humans (29). Rodríguez et al. (2022) found that the area is 12 mm² with a digital caliper in human cadavers, and the ratio is approximately 7% in humans. In this study, it has been observed that the median nerve area of rabbits is approximately 1/9, according to the data of Rodríguez et al. (2022) (28), this ratio is 1/13 the size of humans. The ratio of the median nerve area to the carpal tunnel area in this study was found to be 5% in rabbits and showed similarity with the data of Potuznik et al. (2023) (29). It is thought that the differences in these rates may be due to the measurement method used and population differences.

According to Ginanneschi et al. (2018) (36), it appears that carpal tunnel syndrome can have an effect on the morphology of the Guyon canal as well as the morphology and function of the ulnar nerve at the wrist. The area of the ulnar nerve within the Guyon canal was reported to be 4.54 mm² (4.05–5.11 mm²) on average in humans by ultrasonography (34, 35, 37-39). In this study, it was determined that the area of the ulnar nerve in rabbits was approximately 1/15 of that of humans. According to the data above, the ratio of the ulnar nerve area to the carpal tunnel area is found to be slightly low and 1.7% in this study conducted on rabbits. It is thought that this difference arises from the fact that the Guyon canal is not considered within the carpal tunnel area in humans.

It was reported that the length of the flexor retinaculum, when measured with a digital caliper, was 26.65 mm in humans, 13.03 mm in dogs, 8.46 mm in rabbits, and 2.46 mm in rats (17). The given length of the flexor retinaculum in rabbits is comparable to the length of the superficial flexor retinaculum. In this study, it has been showed similarity with the length in rabbits and was approximately 1/4 that of humans.

With this study, it was determined that there was a narrowing in the area from the carpal tunnel entrance to the inside of the carpal tunnel in rabbits. Although there is a decrease in the areas of the deep and superficial tendons in this region, it is noteworthy that the decrease in the connective tissue is greater. Although there is a proportional increase in the areas of anatomical structures passing through the carpal tunnel, the decrease in the connective tissue tries to compensate this situation. However, the narrowing in the carpal tunnel and the decrease in connective tissue bring the anatomical structures here closer together. This clearly shows that the carpal tunnel is an entrapment neuropathy area, especially for the median nerve.

One of the study limitations is the rabbit cadavers utilized, which had been stored in a formaldehyde solution for an extended duration. Prolonged fixation in formalin may result in secondary shrinkage on soft tissues (40). As a consequence of the subsequent decalcification procedure, tissue detachment occurred, resulting in a reduction in the quantity of histological sections available for analysis.

Conclusions

The carpal tunnel anatomy of rabbits was examined in detail in this study, and the insufficient and erroneous data in previous studies on the carpal tunnel in rabbits were tried to be clarified. In this study, we tried to explain more clearly with the measurements taken that the carpal tunnel is the site of entrapment neuropathy for the median nerve. Additionally, this study emphasizes the validation of the rabbit model in carpal tunnel syndrome research. By establishing the closer resemblance of the rabbit carpal canal to humans, this research paves the way for more accurate investigations into the condition.

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Conflict of interest statement. The authors declare no conflicts of interest.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Anatomske strukture v karpalnem kanalu kunca: primerjava s človekom

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Izvleček: Cilj te raziskave je bil raziskati spremembe v morfologiji in morfometriji anatomskih struktur v karpalnem kanalu, natančneje na mestu ukleščenja medianega živca. Poleg tega smo želeli oceniti primernost kuncev kot živalskega modela za študije karpalnega kanala. V ta namen smo uporabili trupla novozelandskih kuncev in opravili histološki pregled njihovih zapestij. Histološke rezine, obarvane s hematoksilinom in eozinom, smo analizirali s programom ImageJ za pridobitev meritev. Naš pregled je bil osredotočen na tri nivoje karpalnega kanala: vhod, notranjost in izhod. Karpalni kanal je obdan z dvema plastema flektornega retinakuluma na palmarni strani. Znotraj karpalnega kanala smo opazovali globoke in površinske kite upogibalk prstov in radialnih upogibalk zapestja, ne pa tudi tetiv dolge upogibalke. Žilni živčni snopi medianega in ulnarnega živca se nahajata med obema plastema flektornega retinakuluma znotraj karpalnega kanala. Da bi ocenili velikost teh anatomskih struktur, smo izmerili njihove površine na podlagi slik, posnetih na treh prej omenjenih ravneh.

Ob upoštevanju prisotnosti flektornega retinakuluma smo meritev površine karpalnega kanala izvedli tako na vhodu kot znotraj kanala. Pri kuncih zožitev območja od vhoda do notranjosti karpalnega kanala skupaj z zmanjšanjem vezivnega tkiva povzroči, da se anatomske strukture približajo. To zagotavlja jasen dokaz, da je karpalni kanal mesto, kjer se pojavi utesnitvena nevropatija, ki posebej vključuje mediani živec. S primerjavo anatomije karpalnega kanala pri kuncih in psih je bilo ugotovljeno, da so kunci bolj podobni ljudem, zlasti v smislu flektornega retinakuluma. Ta raziskava poudarja pomen uporabe kunčjega modela za preučevanje sindroma karpalnega kanala, saj prikazuje podobnosti med kunčjo in človeško anatomijo ter poudarja pomen tega živalskega modela za prihodnje preiskave.

Ključne besede: karpalni kanal; morfometrija; histologija; mediani živec; ulnarni živec; flektorni retinaculum