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Microanatomic evaluation of the axon number and the parenchyma/stroma ratio of the sciatic and tibial nerves during human fetal anatomical development

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Abstract

Aim: Determination of the axon numbers of nerve fibers and evaluation of the parenchyma/stroma ratios to indicate the density of the periaxonal tissue (myelin sheath and periaxonal connective tissue) were aimed by using stereological methods on the histological sections obtained from the sciatic and tibial nerves of the human fetuses.

Materials and Methods: This histomorphometric study was carried out on 20 aborted human fetuses (14 males, 6 females, between 12 and 38 gestational weeks) with no external anomalies or malformation. Peripheral nervous tissue specimens harvested from the three levels [proximal (sciatic nerve), middle (first part of the tibial nerve), and distal (distal part of the tibial nerve) levels] through the lower extremities, by anatomical dissection, on either side. Histological sections were prepared. Digital images of the sections were used for the stereological study.

Results: The results showed (I) the axon number of peripheral nerves increases as the fetal period progresses; (II) the axon number of peripheral nerves do not change from proximal to the distal specimen levels; (III) the axon number of peripheral nerves increases at the same rate throughout the nerve; (IV) the parenchyma/stroma ratio of peripheral nerves does not change along with the nerve, during the fetal period; (V) the parenchyma/stroma ratio of the peripheral nerves does not vary between specimen levels.

Conclusion: Our results may contribute to the experimental and clinical research primarily related to sciatic and tibial nerves and the histological interpretation of fetal autopsy materials in fields such as fetopathology and forensic medicine.

Introduction

The nervous system develops during the prenatal period and early postnatal period. Myelination begins in the twentieth week of the prenatal period [1]. The development continues in the postnatal period, and maturation is completed in the adult period. During the fetal period, many causes such as genetic and environmental factors, pre-eclampsia, placental insufficiency, and smoking may adversely affect the axon development and the myelination in the peripheral nervous system [1-3].

Some of the neurological diseases involving the peripheral nerves are characterized by symptoms such as weakness in the distal parts of the extremities. Different histological features of the proximal and the distal portions of the peripheral nerve may help to understand the physiopathology for the diagnosis and treatment of the polyneuropathies [4, 5]. The rate of nervous system development in the fetus is high from the 5th month of the prenatal period to the 6th postnatal month. Problems in this period can also cause developmental defects in the nervous system. Hereditary nervous system diseases may be presented with similar histopathological alterations [6]. However, peripheral neuropathies can also occur due to pressure or compression, systemic diseases, and infections [7].

In the literature, studies on the sciatic nerve (SN) and its branches of the human fetuses were often related to macroscopic morphometric analysis and variations [8, 9]. Also, research on the prenatal nerve fibers of the peripheral nervous system focused on experimental animals [10-12]. In some experimental studies covering the prenatal, neonatal, and adult periods, the axon numbers of SN have been investigated in terms of development or under some phar-

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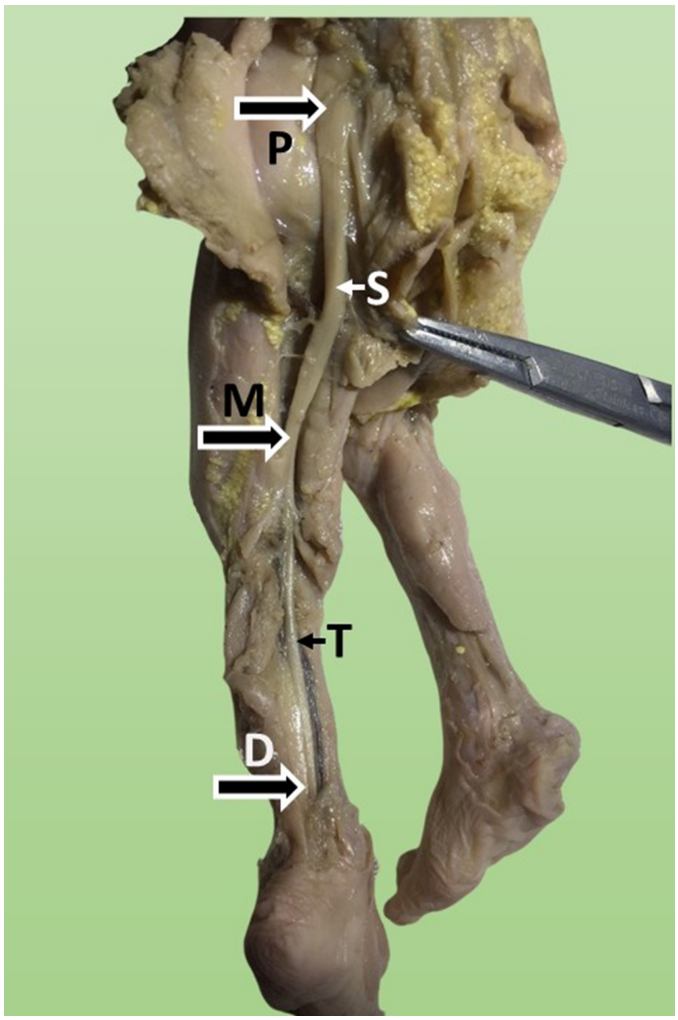


Figure 1. Photograph showing the dissection of a 15-week-old male fetus. View of the sciatic nerve and tibial nerve (P: Proximal Sample Level, M: Medium Sample Level, D: Distal Level, S: Sciatic Nerve, T: Tibial Nerve).

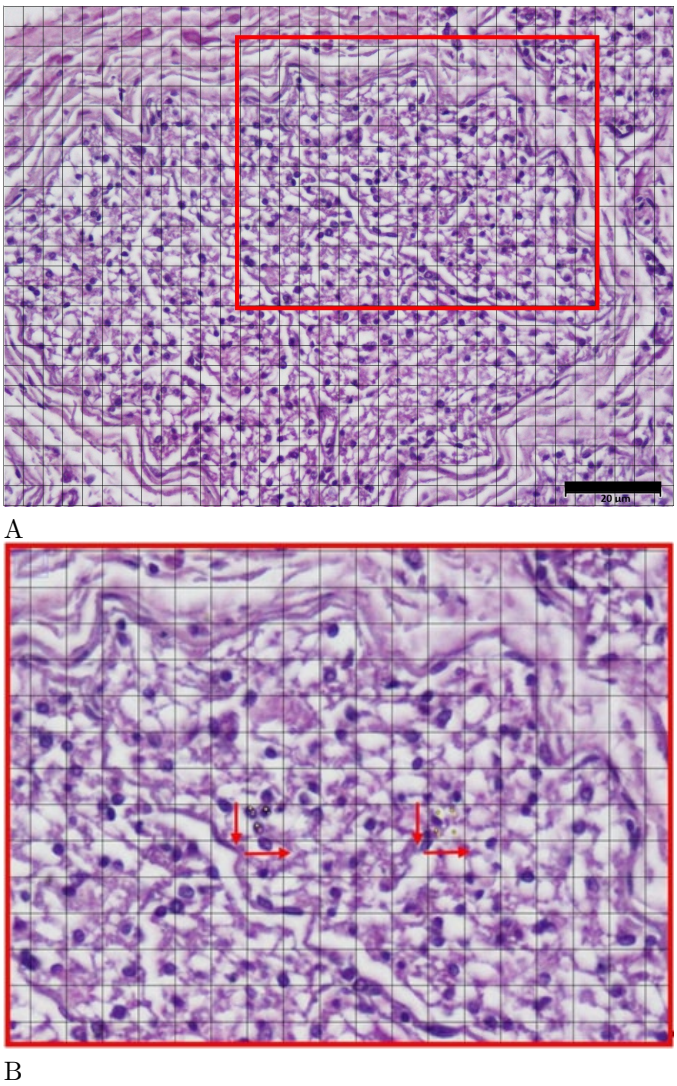


Figure 2. A: Micrograph showing the axon counting scene on ImageJ. Cross section of the Sciatic Nerve with an unbiased counting frame (grid) superimposed on it [Field of view (FOV)]. (22-week old male fetus. Scale bar:20µm. Grid:25x34. Hematoxylin and eosin staining (H&E). x480. Resolution: 1600x1200 px.). B: Area in rectangle was digitally magnified in purpose of presentation.

macological factors by stereological methods [3, 10].

In peripheral nerve tissue, axons are defined as the parenchymal region, and the periaxonal tissue (myelin sheath and periaxonal connective tissue) is defined as the stromal region. In the literature, there are many studies regarding the number of axons in the prenatal period in human fetuses.

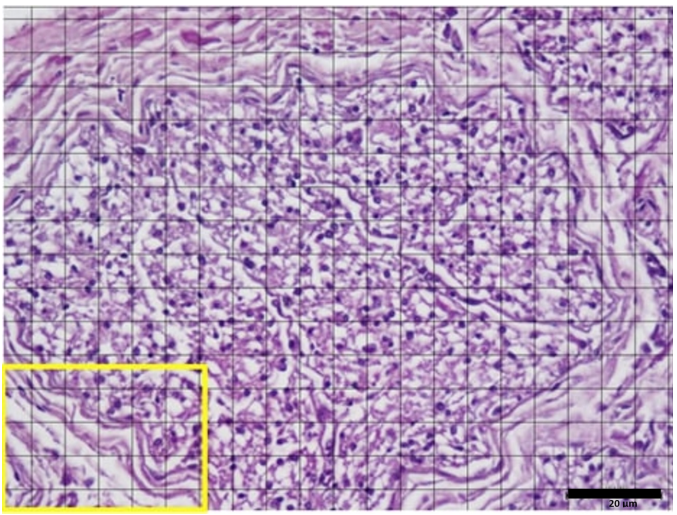
This study aimed to quantitatively analyse the parenchyma/stroma ratios to indicate the density of the periaxonal tissue (myelin sheath and periaxonal connective tissue) besides the determination of axon numbers of nerve fibers per unit area by using stereological methods on the histological sections obtained from the SN and tibial nerves (TN) of the human fetuses. To the best of our knowledge, this is the first report quantitatively analyzing the density of the periaxonal stromal tissues of a peripheral nerve in the prenatal period in human fetuses.

Materials and Methods

This study is carried out on 20 aborted human fetuses (14 males, 6 females) fixed in 10% formalin, aged between 12 and 38 weeks (w) of gestation. All were spontaneous abortions or stillbirths and neonatal deaths (died owing to premature or prenatal asphyxia). Cases that did not have any external pathology or anomaly included in the study.

In this study, non-fresh formalin-fixed necropsy samples that were obtained from the fetus collection of the department were used. Written consents from the families were collected, and approval from the Institutional Ethics Board was obtained before the commencement of the study (İzmir Katip Çelebi University, Non-Interventional Clinical Research Ethics Committee, Date: 22.06.2017, Decision number: 116). Our research has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all subsequent revisions.

The gestational age of the fetuses was determined by their crown-rump length (CRL), Biparietal Diameter (BPD),



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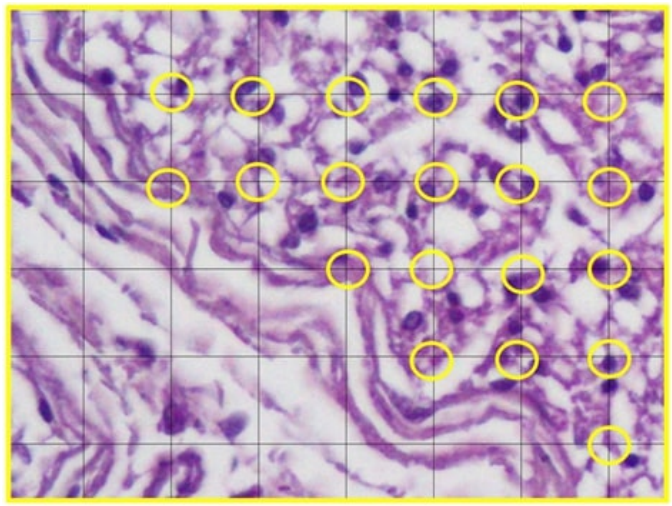

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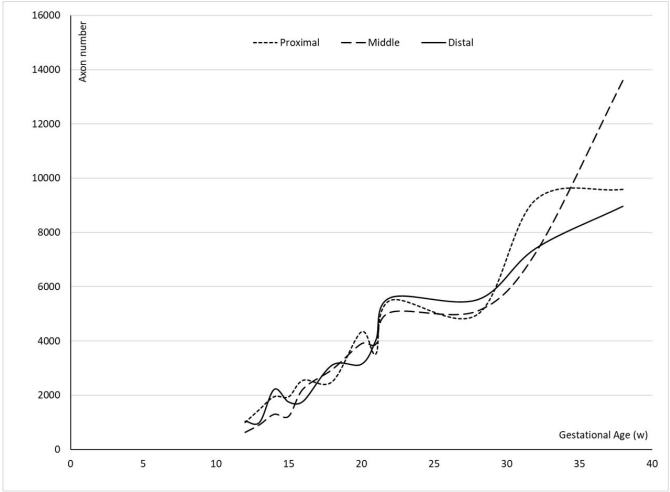
Figure 3. Micrograph showing the determination scene of the parenchymal and stromal tissue components on ImageJ. Yellow circles show the evaluation points. Cross section of the Sciatic Nerve with a grid superimposed on it [Field of view (FOV)]. (22-week-old male fetus. Grid:15x20. Hematoxylin and eosin staining (H&E). x480. Resolution: 1600x1200 px. Scale bar: 20µm). B: Area in rectangle was digitally magnified in purpose of presentation.

Head Circumference (HC), Femur Length (FL) and Foot Length (PL). Parameters were measured with a caliper, tape measure, or ruler [1].

Dissection

Deep anatomical dissection of the posterior compartments of the gluteal, thigh, and leg regions on both sides of the fetuses was performed. SN and its branches, TN, and common fibular nerve were exposed along the lower limb (Figure 1). Then tissue specimens were harvested from three different levels (proximal, middle, and distal levels) for the preparation of sections for further microscopy (Figure 1). The proximal level tissue specimen was harvested approximately 5 mm in length from the first part of SN that came



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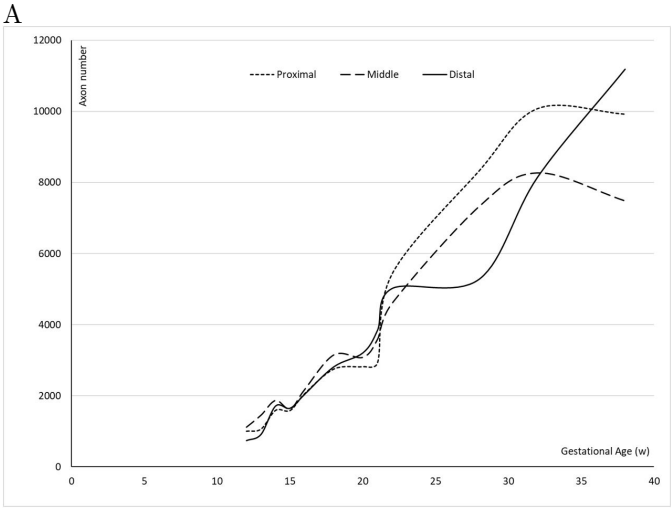

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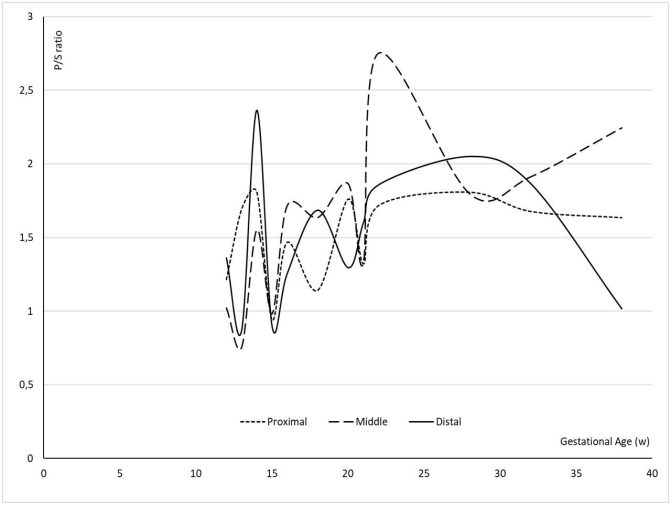
Figure 4. Axon numbers of proximal, middle, and distal levels during the fetal period A: Right side. B: Left side.

out from just below the piriformis in the gluteal region. The middle level tissue specimen was harvested approximately 5 mm in length from the first part of TN immediately after branching from SN in the popliteal region. The distal level tissue specimen was harvested approximately 5 mm in length from the distal part of TN just above the flexor retinaculum on the posteromedial of the ankle.

Histologic procedure

For the histological study, the tissue specimens (proximal, middle, and distal levels) were processed using an automated tissue processor (Leica TP1020, Leica Microsystems, Germany), and the paraffin embedding technique (Leica EG 1150C, Leica Microsystems, Germany) was carried out. Tissue specimens placed in a vertical position and cut-side down into the embedding mold then sectioned at 5 µm with a microtome (Leica RM2245, Leica Microsystems, Germany) and stained with hematoxylin-eosin (H&E) so at least 3 section is prepared. For optical microscopy, images of each section were captured using an Olympus DP21 (Olympus, Tokyo, Japan) digital microscopy camera system, with a magnification of 40X objective was used (Figure 2A, Figure 3A, Resolution: 1600x1200 px).



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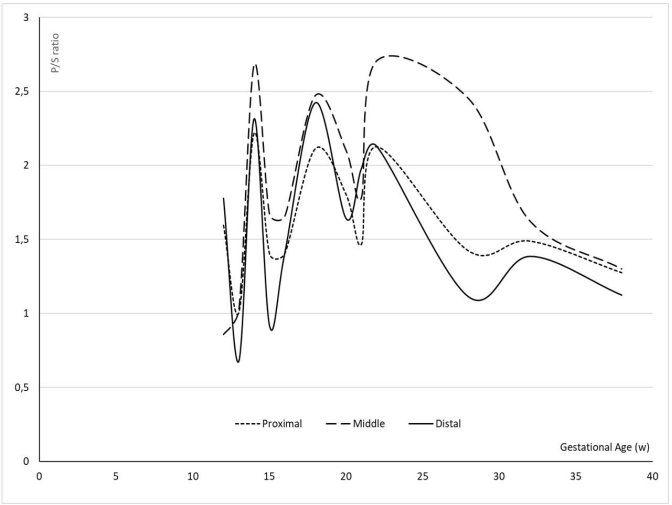

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Figure 5. Parenchyma/Stroma (P/S) ratios of proximal, middle, and distal level samples during the fetal period A: Right side. B: Left side.

Measurement procedure

In the literature, axons are defined as "parenchyma" and periaxonal region (myelin sheath and periaxonal connective tissue) as "stroma" in the peripheral nerve tissue. In our study, these parenchymal and stromal structures were examined quantitatively. Sections prepared from each specimen level were examined for both axon number and proportional composition of parenchymal and stromal structures. Previously used methods and classical stereological perspective methods were used [10, 13-15]. The image area, which is covered with the grid in section images and used for analysis, is defined as "field of view (FOV) (the unit area)". FOV was an area of 11016 μm^2 (Figure 2A, Figure 3A). ImageJ software (Rasband, WS, ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018) was used in counting. For the axon count and parenchyma/stroma ratio calculation, grid alternatives (10x15, 15x20, and 25x34 cells) were prepared for the efficient count on FOV. Grids were created and placed over the FOV by ImageJ.

Preliminary count results obtained from images were compared. The interobserver variability was evaluated. Since the interobserver variability was not found significant, the 25x34 cell grid and the 15x20 cell grid were preferred for the axon count and the parenchyma/stroma ratio calculation, respectively.

While counting axons, an "unbiased counting frame" was used following the stereological principles to calculate the axon numbers in the grid cells (Figure 2) [13, 15, 16]. Grid cells for axon counting were determined by the systematic random sampling method. To determine the average number of axons in the unit area (FOV), counts were randomly performed in 3 different regions in each histological section (Figure 2). Three hundred intersection points of the grid (15X20 cell) superimposed to the FOV evaluated for parenchyma/stroma ratio. Points hitting the parenchyma (axonal region) and the stroma (myelin sheath and periaxonal connective tissue) are counted (Figure 3). Then the ratio is calculated. For determining the average parenchyma/stroma ratio in the unit area (FOV), counts were randomly performed in 3 different regions in each histological section (Figure 3).

Since fetal growth is considered as a factor during the fetal period, the axon number which is obtained by using a static unbiased counting frame of the fetus(es) of a particular week, was corrected by a coefficient. The coefficient calculation was based on the FL (Table 1). The axon number of FOV was multiplied by the fetal growth correction factor $[F_{fgc} = (\frac{\overline{FL}_n}{\overline{FL}_{min}})]$. So the corrected axon count is calculated as:$AN_{cor} = AN_n \times (\frac{\overline{FL}_n}{\overline{FL}_{min}})$ where AN_{cor} is the corrected axon number, AN_n is the axon number of the fetus(es) of a week, $\overline{FL}_n$n is the average femur length of the same week and $\overline{FL}_{min}$min is the average femur length of the fetus of the lowest week (Table 1).

Statistical analysis

All statistical procedures were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Cases were grouped based on the gestational age as Group 1 (12-20 weeks), Group 2 (21-29 weeks), Group 3 (30-38 weeks). The data set was tested for normality with Shapiro–Wilk Test. Descriptive statistics of the parenchyma/stroma ratios and the axon numbers by the gestational weeks, the gestational age groups, the specimen levels, and the sides calculated. Normally distributed data summarized as mean (SD). Non-normally distributed data summarized as median (min-max). The data obtained were compared between the sides, gestational age groups and the specimen levels. Depending on the features of the data and the comparison of the data sets, Student-T Test, One-Way ANOVA (post hoc Scheffe Test), paired samples T Test, Wilcoxon Test, Kruskal-Wallis, and Friedman Test and Pearson Correlation Test were used where appropriate and showed in table legends. Significance levels were set at a p-value of <0.05

Results

The morphometric growth parameters (CRL, BPD, HC, FL, PL) of 20 fetuses (14M, 6F) aged between 12 and 38 gestational weeks for gestational age determination are

Table 1. The morphometric growth parameters of the fetuses (mm).

No	Age (w)	Gender	CRL	HC	BPD	FL	PL
1	12	M	97	91	24	25	15
2	13	M	105	107	26	27	15
3	14	F	134	136	39	34	20
4	15	M	128	140	36	37	24
5	16	M	139	153	37	39	26
6	16	M	137	138	37	38	26
7	16	M	142	150	40	44	27
8	18	M	165	180	40	52	33
9	18	M	163	175	44	44	29
10	20	M	193	191	46	52	38
11	20	M	201	203	55	56	38
12	20	F	183	186	42	50	36
13	21	F	210	213	49	55	42
14	21	M	226	212	52	57	42
15	22	F	221	219	43	54	39
16	22	M	200	234	61	61	46
17	28	F	261	288	78	74	59
18	32	M	304	334	88	93	71
19	32	F	315	319	89	80	70
20	38	F	368	375	106	98	82

CRL: Crown-Rump length, HC: Head Circumference, BPD: Biparietal Diameter, FL: Femur Length, PL: Foot Length.

shown in Table 1. Means of the axon numbers of the specimen levels (proximal, middle, and distal levels) and the gestational weeks of the fetuses on both sides are shown in Table 2.

There were no statistically significant differences in all compared parameters between sexes (Student T Test, $P>0.05$). At each specimen level, there were no significant differences in the axon number between the sides (Paired Samples T-test, $P>0.05$, Table 2). The axon numbers of the proximal, middle, and distal specimen levels during the fetal period on the right and left sides are shown in Figure 4 and Figure 5, respectively. There were no significant differences in the axon number between the three specimen levels on both sides (One Way ANOVA, $P>0.05$, Table 2). For both sides, the axon numbers at each specimen level for the gestational age groups are shown in Table 3. There were significant differences between all gestational age groups (One-Way ANOVA, Post-Hoc Scheffe, $P<0.001$, Table 3). There were no significant differences between the specimen levels (proximal, middle, distal) on either side (One-Way ANOVA, $P>0.05$, Table 3). The axon numbers of the specimen levels on either side correlated well and positively with the gestational age (w) (for each parameter $R>0.9$, $P<0.001$).

Mean parenchyma/stroma ratios were calculated for each gestational age group, side, and specimen level (Table 4). There was no significant difference between the sides for parenchyma/stroma ratios (Wilcoxon Test, $P>0.05$, Table 4). The parenchyma/stroma ratios of the proximal, middle, and distal specimen levels during the fetal period on the right and left sides are shown in Figure 6 and Figure 7, respectively. There were no significant differences between the specimen levels (proximal, middle, distal) on either side (Friedmann Test, $P>0.05$, Table 4).

For both sides, parenchyma/stroma ratios at each specimen level for the gestational age groups are shown in Table 5. For the parenchyma/stroma ratio, there was no difference between the gestational age groups (Kruskal-Wallis Test, $P>0.05$), between the specimen levels (Friedmann Test, $P>0.05$), and between the sides (Wilcoxon Test, $P>0.05$) (Table 5). There was no correlation between the gestational age (w) and the parenchyma/stroma ratio ($P>0.05$).

Discussion

Detailed knowledge about the development of the nervous system in the prenatal period may help to understand both functional and clinical aspects of the diseases in the postnatal period. Literature shows the importance of this knowledge besides the value of the changes in the amount of the parenchymal and stromal components of the tissues, in many diseases [6, 17-20].

Demyelination and axonal damages of peripheral nerves in some diseases like Guillain-Barre Syndrome, Chronic inflammatory demyelinating polyneuropathy, diabetic peripheral neuropathy is valuable for researchers. In hereditary diseases, gene mutations can cause demyelinating or axonal pathologies in the peripheral nerves. Clinical features in peripheral neuropathies caused by drugs, environmental toxins, and vasculitis are distinguished in the distal parts of the extremities. It is understood from such situations that the physiopathological features may differ in the proximal and distal parts of the extremities in terms of peripheral nerves. In such diseases, axonal damage, histopathological alterations in the myelin sheath, and periaxonal connective tissue are observed [6, 18-21].

Neuromuscular disorders and neuropathies affecting the fetus are observed with clinical problems in the neonatal period and childhood [22]. Hereditary peripheral neuropathies are a group of hereditary diseases of the nervous system. Schwann cell-axon interactions formed by the functions of genes causing hereditary neuropathies have an important role in clinical pathology [21]. Along with its molecular basis and neurophysiological findings, fetal histomorphological features can also improve our understanding of hereditary peripheral neuropathies [23]. Therefore, in our study, it is hoped that describing the histological features of axonal and periaxonal structures in the peripheral nerves in detail for the prenatal period may support clinical research.

SN shows the same basic features as all spinal nerves in terms of embryological development [1, 24]. The myelination process is also an important component in peripheral nerve development. The myelin sheath in the spinal cord begins to form in the fetal period, and the formation continues during the postnatal first year [1, 3]. Myelin is formed by Schwann cells in the peripheral nervous system. In some respects, these fundamentals explain axon number, myelination, parenchymal and stromal components in peripheral nerve tissues, and developmental anatomy of peripheral nerves. In the literature, the axon number is accepted as one of the basic parameters in peripheral nerve development [10, 12, 16].

Table 2. Means of the axon numbers of the specimen levels (proximal, middle, and distal levels) and the gestational weeks of the fetuses on both sides.

		Proximal† (SN)		Middle† (TN-proximal)		Distal† (TN-distal)	
Age (w)	Gender (N)	Right*	Left	Right*	Left	Right*	Left
12	M (1)	1003	1003	629	1122	1037	739
13	M (1)	1491	1064	915	1461	1003	915
14	F (1)	1954	1591	1295	1874	2211	1712
15	M (1)	1949	1587	1225	1635	1743	1650
16	M (3)	2560	2061	2237	2183	1775	2043
18	M (2)	2509	2753	2949	3156	3109	2815
20	M (2), F (1)	4342	2819	3895	3087	3137	3208
21	F (1), M (1)	3532	2929	3880	3596	4029	3846
22	F (1), M (1)	5503	5428	5053	4605	5594	5018
28	F (1)	4985	8335	5103	7335	5517	5284
32	M (1), F (1)	9211	10 076	7251	8271	7408	8156
38	F (1)	9592	9918	13 602	7483	8961	11 184

SN: Sciatic Nerve, TN: Tibial Nerve

*P>0.05, Paired Samples T-Test. No significant differences between the sides (R-L)

†P>0.05, One-Way ANOVA. No significant differences between the specimen levels (proximal, middle, distal) on either side.

Table 3. Mean axon numbers of the specimen levels (proximal, middle, and distal levels) by age groups and sides [Mean (SD)].

		Proximal‡ (SN)		Middle‡ (TN-proximal)		Distal‡ (TN-distal)	
Age group(w)	N	Right†	Left	Right†	Left	Right†	Left
Group 1* (12-20)	12	2677 (1155)	2116 (747)	2363 (1198)	2351 (792)	2246 (876)	2200 (963)
Group 2* (21-29)	5	4611 (1139)	5010 (2277)	4594 (1501)	4747 (1612)	4952 (845)	4602 (943)
Group 3* (30-38)	3	9338 (1388)	10 023 (825)	9368 (3917)	8008 (1227)	7926 (1215)	9165 (1811)
Total (12-38)	20	4159 (2631)	4025 (3118)	3972 (3042)	3799 (2330)	3774 (2305)	3845 (2720)

SN: Sciatic Nerve, TN: Tibial Nerve

*P<0.001, One-Way ANOVA, Post-Hoc Scheffe. Significant differences between all age groups.

†P>0.05, Paired Samples T-Test. No significant differences between the sides (R-L)

‡P>0.05, One-Way ANOVA. No significant differences between the specimen levels (proximal, middle, distal) on either side.

To the best of our knowledge, no microscopic studies have been conducted on the composition of periaxonal structures and axon numbers related to SN and TN in human fetuses in the prenatal period. In our study, the axon number and parenchyma/stroma ratio (density of the periaxonal connective tissue and myelin sheath) determined in histological sections of peripheral nerves of the SN and TN in human fetuses. With our study, it was aimed to discuss the parenchyma/stroma ratio examination as an element while evaluating the development of the peripheral nervous system in the fetal period.

In our study, it was determined that the number of axons in the unit area in all three specimen levels increased as the gestational week progressed. Also, there was a positive correlation between the gestational age and the number of axons ($R>0.9$, $P<0.001$). In terms of axon numbers, our findings are consistent with the literature on peripheral nervous system development [2, 3]. Table 3 shows that the difference of means of the axon numbers between Group 2 (20-29 w) and Group 3 (30-38 w) is higher than that between Group 1 (12-20 w) and Group 2 (20-29 w). This result is accordant with the high progress rate of the organ maturation of the prenatal 3rd trimester. Moore, Persaud [1] and Altunkaynak, Altunkaynak [3], in their experimental study in rats, demonstrated similar findings regarding the change in the axon numbers in the same period, which represents the one in humans.

Our findings showed that, on either side, from proximal to the distal direction the number of axons per unit area did not change ($P>0.05$, Table 3). It is noteworthy that as gestational age progresses, the number of axons of each section level increases at the same rate. This observation is interpreted that in the lower extremities developing rate of peripheral nerve tissue is the same at proximal, middle, and distal levels.

Peripheral neuropathies can be classified as axonal and demyelinating. Insults that directly injure the axon result in axonal neuropathies. Myelin loss accompanies axonal degeneration. A decrease in the density of axons (parenchymal component) is the feature of the axonal neuropathies. Demyelinating neuropathies are demonstrated with rela-

Table 4. Parenchyma/stroma ratios of the specimen levels (proximal, middle, and distal levels) and the gestational weeks of the fetuses on both sides (Median)

		Proximal Level† (SN)		Middle Level† (TN-proximal)		Distal Level† (TN-distal)	
Age (w)	Gender (N)	Right*	Left	Right*	Left	Right*	Left
12	M (1)	1.22	1.60	1.02	0.86	1.36	1.78
13	M (1)	1.69	1.01	0.75	1.03	0.87	0.68
14	F (1)	1.81	2.22	1.55	2.68	2.36	2.32
15	M (1)	0.94	1.40	0.98	1.67	0.90	0.92
16	M (3)	1.47	1.40	1.72	1.66	1.26	1.41
18	M (2)	1.14	2.11	1.64	2.47	1.69	2.43
20	M (2), F (1)	1.76	1.80	1.87	2.10	1.30	1.64
21	F (1), M (1)	1.32	1.47	1.33	1.78	1.60	1.98
22	F (1), M (1)	1.72	2.13	2.75	2.71	1.86	2.13
28	F (1)	1.81	1.42	1.80	2.45	2.05	1.11
32	M (1), F (1)	1.68	1.49	1.91	1.63	1.87	1.39
38	F (1)	1.64	1.27	2.24	1.30	1.02	1.12

SN: Sciatic Nerve, TN: Tibial Nerve

*P>0.05, Wilcoxon Test. No significant differences between the sides (R-L).

†P>0.05, Friedmann Test. No significant differences between the specimen levels (proximal, middle, distal) on either side.

Table 5. Parenchyma/stroma ratios of the specimen levels (proximal, middle, and distal levels) by age groups and sides [Median (Min-Max)].

		Proximal (SN)		Middle (TN-proximal)		Distal (TN-distal)	
Age group(w)	N	Right	Left	Right	Left	Right	Left
Group 1* (12-20)	12	1.43 (0.94-2.00)	1.55 (0.75-1.82)	1.36 (0.87-2.36)	1.47 (1.01-2.22)	1.67 (0.86-2.68)	1.51 (0.68-3.08)
Group 2* (21-29)	5	1.67 (0.95-2.37)	1.79 (1.32-3.05)	1.60 (1.17-2.05)	1.654 (1.11-2.53)	2.46 (1.15-2.88)	2.056 (0.99-2.68)
Group 3* (30-38)	3	1.64 (1.59-1.76)	2.24 (1.49-2.33)	1.72 (1.02-2.02)	1.48 (1.27-1.50)	1.32 (1.30-1.93)	1.208 (1.12-1.56)
Total (12-38)	20	1.65 (0.94-2.37) [a,x]	1.49 (0.75-3.05) [a,y]	1.74 (0.87-2.36)[b,x]	1.92 (1.01-2.53)[b,y]	1.54 (0.86-2.88) [c,x]	1.54 (0.68-3.08)[c,y]

SN: Sciatic Nerve, TN: Tibial Nerve

*P>0.05, Kruskal-Wallis. No significant differences between all age groups, for each level.

[a,b,c]P>0.05, Wilcoxon Test. No significant differences between the sides (R-L).

[x,y]P>0.05, Friedmann Test. No significant differences between the specimen levels (proximal, middle, distal) on either side

tively spared axons and demyelination occurs discontinuously [6]. In terms of tissue development, the ratio of parenchyma and stroma is valuable to follow the function of the tissue and the development process. Kaemmer, Bozkurt [17] emphasized that it is important to objectively define the change of tissue component ratios in the peripheral nerves. Additionally, it is stated that considering such ratios for a therapeutic approach to peripheral nerve pathologies can be used as a prognostic marker [17].

In our study, the parenchyma/stroma ratios in the histological sections of SN and TN were determined in the specimens harvested from the proximal level (mean:1.56), middle level (mean:1.75) and distal level (mean:1.54) regions during the fetal period (Table 4, Table 5).

Table 4 and Table 5 show no significant difference between the sides as well as specimen levels in terms of parenchyma/stroma ratio. When fetal age groups were compared, it was observed that the parenchyma/stroma ratio did not alter (P>0.05, Table 5), and the mean of ratio in the fetal period was determined as 1.62. Our results suggest that along with the lower extremity and during the fetal period, parenchymal and stromal components of the SN and TN develop at the same rate. These findings seem to be important for the histopathology and the anatomy of the human fetus. To our knowledge, this histomorphometric ratio of the SN and TN has not been previously described or discussed in the literature.

The limitations of this study are that the samples included in the study were in utero mort, they have exposed to formaldehyde solution for a long time, and the number of human fetuses in both sexes was low. We believe that the development of the peripheral nervous system in the fetal period can be better explained by studies on a higher number of fetuses. The histological procedures for the fresh tissues could not be applied to the samples because they are not harvested immediately after the abortus. Due to the research material is not fresh samples, more detailed microscopic examination (special staining methods for connective tissue and the reticulum fibers contained therein)

or advanced techniques like immunohistochemistry or electron microscopy could not be performed.

Conclusion

In conclusion, our findings demonstrated that as gestational age progresses, the axon numbers on the histological sections of SN and TN increases, and don't alter between the proximal, middle, and distal levels due to the same increase rate at each specimen levels. Parenchyma/stroma ratio does not vary between the sides and specimen levels, during the fetal period. This study should be considered as a preliminary study that quantitatively evaluates the development of the peripheral nervous system in parenchyma/stroma ratio in dead human fetuses. Further experimental or in vivo research may demonstrate the change of axon number and parenchyma/stroma ratios related to peripheral nervous system diseases. We hope that our results will contribute to the experimental and clinical research especially related to SN and TN and the histological interpretation of fetal autopsy material in fields such as fetopathology and forensic medicine.

Ethics approval

Ethical approval for this study Izmir Katip Celebi University Retrieved from the Non-Invasive Clinical Research Ethics Committee (Date: 22.06.2017, Decision number: 116).

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