Neuropathic Changes of Osmicated Common Peroneal and Tibial Nerve in Diabetic Patients and the Related Risk Factors

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Abstract

So much studies done to present the diabetic neuropathic changes of the peripheral nerves but most of them were done experimentally on laboratory animals , but little about human diabetic neuropathic and structural changes specially of large nerves like common peroneal and tibial nerve .Accordingly the present work designed to study the neuropathic changes of the tibial and common peroneal nerve samples, taken from amputated legs of 30 diabetic patients (8 females and 22 males) at age ranging between (55-75) years, and from 30 cadavers (4 females and 26 males) at age ranging between (25-50) years as control group. The teased and osmicated tibial and common peroneal nerve fibers, and histological sections taken from diabetic patients showed different deformity and Morphometrical changes, of that ; paranodal swelling, segmental demyelination, some areas with an areas of un even myelin and shortest segmentation the (common peroneal nerve 22µ and tibial nerve 265µ) in comparison to the control group. The multiple regression analysis, showed significant effect of age of the patient and duration of diabetes on the neuropathic changes.

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Introduction

So much studies done to present the diabetic neuropathic changes of the peripheral nerves but most of them were in the experimental animals [1-2], but little about human diabetic neuropathic and structural changes specially large nerves like common peroneal and tibial nerve because it seems impossible to take such nerve sample from living human being specially from diabetic patients .

One of the most common complications of diabetes affecting the lower limbs is diabetic neuropathy [3]. Diabetic neuropathy is a common disorder, defined as signs and symptoms of peripheral nerve dysfunction in a patient with diabetes mellitus [4]. The pathogenesis of diabetic neuropathy is complex; chronic hyperglycemia is a major factor to induces peripheral nerve fibers injury and may be autonomic neuropathies [5].

It was mentioned by Aaberg et al.[6]; that male patients with type (2) diabetes may develop diabetic polyneuropathy earlier than female patients, and may effected by age severity and duration of diabetes.

The relationship among Schwann cells, axons, and the perineurial barrier emphasize

the key role in normal functions of the nerve [7].

Behse et al. [8] studied morphological findings in sural nerves biopsy specimens obtained from middle-aged and elderly diabetics suffering from neuropathy and they found that myelinated nerve fiber density, especially large fibers, was decreased, segmental and demyelination were the most prominent myelin alteration in teased fibers which were found in only a few fibers. The severity and duration of diabetes may be a factor in the decrease of myelinated fiber density. Large unmyelinated nerve fibers were also decreased and the mean diameter of unmyelinated fibers was reduced. Axonal degeneration and Schwann cell damage seem to proceed independently of each other.

The Aim of The Study:

This study is carried out to fulfill the following aims:

1. Determine the normal un diabetic Morphometrical measurement of the osmium stained and teased common peroneal and tibial nerves.
2. Determine the neuropathic changes of the common peroneal and tibial nerves in diabetic patients'
3. Evaluate multiple risk factors for the diabetic peripheral neuropathy.

Patients and Methods

The samples of tibial and common peroneal nerve taken from amputated legs of (30) diabetic patients (22 male and 8 female at age ranging between 55–75 years) who were subjected to the amputation of their legs from above the knee, in the surgical theater of different hospitals in Erbil city. Other samples taken from (30) cadavers (4 female and 26 male, their age varying between 25–50 years) newly imported to the forensic medicine as control group.

In the theater; the amputated leg was taken to a side room with the anterior surface on the dissecting table. The skin incised down the middle of the popliteal fossa to posterior leg.. Fat removed from the fossa with probe and forceps and the remnant of the deep fascia was removed to expose the medial and lateral head of gastrocnemius. By using blunt dissection the common peroneal nerve is followed laterally along the superolateral border of the popliteal fossa. The common peroneal nerve parallels the biceps femoris tendon and passes superficial to the lateral head of gastrocnemius muscle [9], at this site two centimeters from the nerve was taken. The tibial nerve followed down into the popliteal fossa and continued inferiorly posterior to the popliteal vein most superficial to the large neurovascular structures, as it enters the fossa it inclines lateral to the vessels, then crosses posteriomedially to the vessels [10], the nerve separated from the loose connective tissue that surrounded it, followed inferiorly, deep to the plantaris and gastrocnemius muscles at the inferior border of popliteal fossa [9]. The index fingers inserted between the two bellies of the gastrocnemius muscle, the muscle bellies pulled apart for a distance of about 5–10 cm to expose the part of it that pass to the leg. At this site two centimeters were taken from the tibial nerve. The nerve specimens were washed with normal saline, plotted dried on a filter paper for the blood to be absorbed, cleaned from the surrounding connective tissue and divided into two parts one cm for nerve teasing and the other for tissue processing.

The Limitations of The Study:

There was a difference between the age groups in the diabetic and control for the nerve samples because of the difficulty in collecting same age group samples.

Statistical Analysis

Statistical analysis was made using statistical package for social sciences (SPSS) computer software version 15. The following tests were

used: ANOVA test, independent samples t test, simple linear correlation and multiple regression analysis. A p value of ≤ 0.05 was considered as statistically significant.

Results

The Results of The Nerve Teasing Fibers of The CPN And TN

The mean diameter of the paranodal area of both common peroneal nerve (CPN) and tibial nerve (TN) in the control group was 8µ (figure 1-2), while in the diabetic group the mean paranodal area in CPN was 19 µ and in TN was 22µ, which indicated for significant paranodal swelling in the diabetics as shown in (Figure 3).

Regarding the length of the intermodal segments of the diabetic samples was 22µ and that of the TN was 265µ showed shorting with a shortest segment of the diabetic. Segmental demyelination, irregularity and thin myelin or some segments lacking myelin completely, frequent degenerated nerve fibers, and shortening of internodal length were detected (figure 2). The control un diabetic samples group appeared difficult to measure the length of the internodal segments of the nerve fiber.

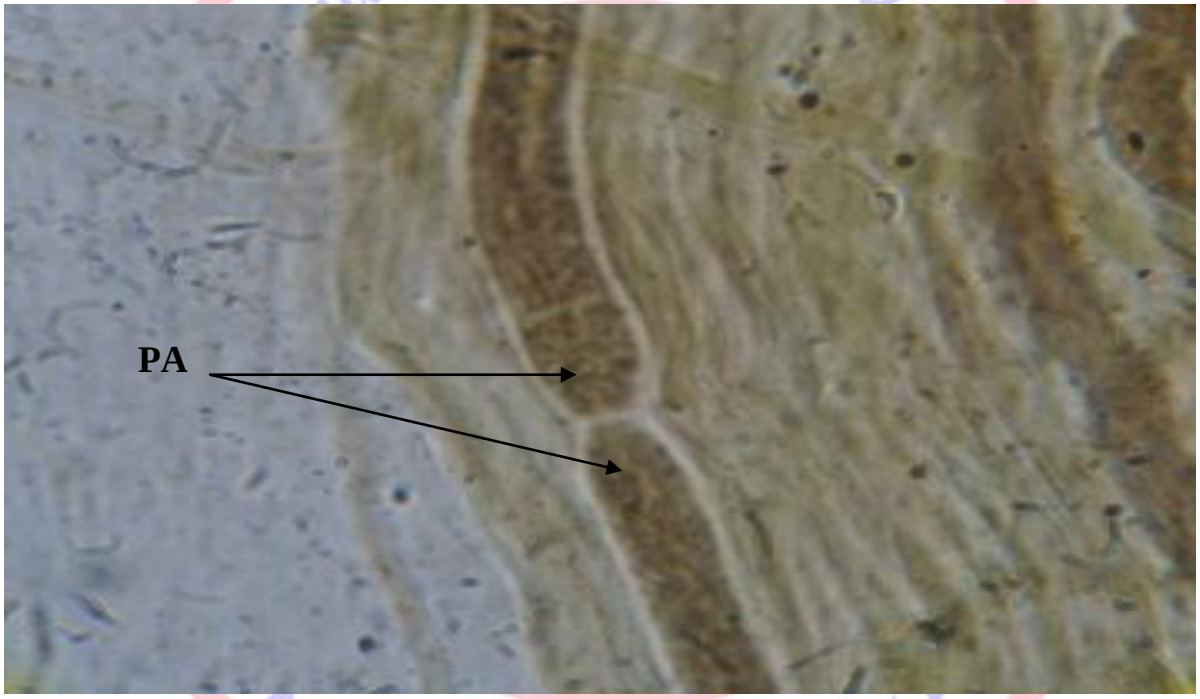

Figure (1): Teased TN fiber from a normal subject (PA =Paranodal area). X 1000.

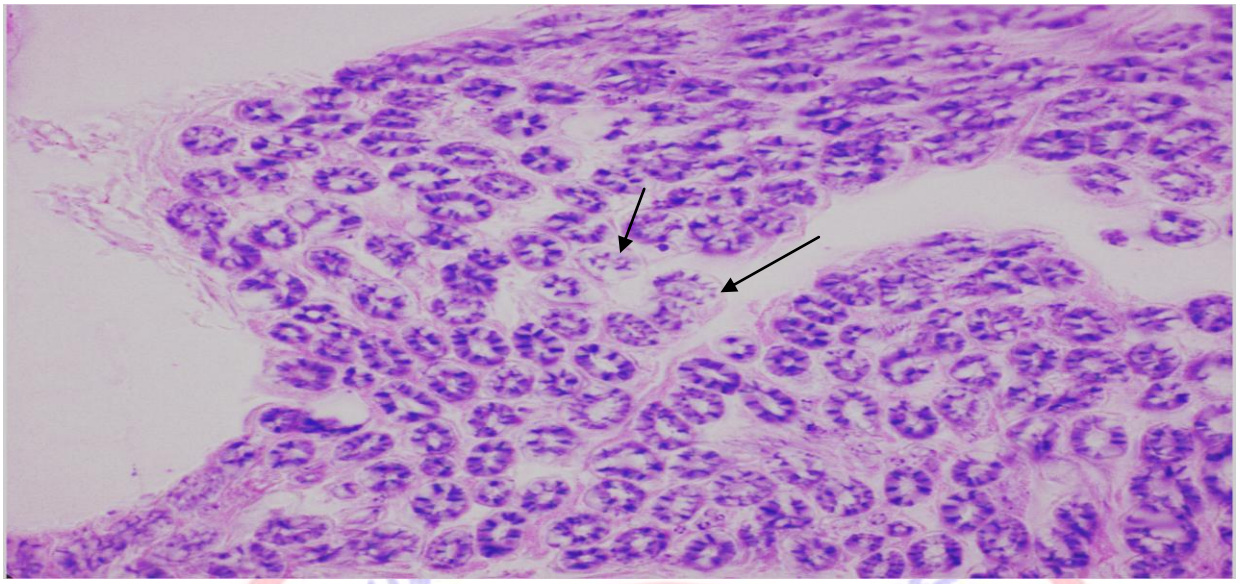

Figure (2): Cross section from TN normal appearance of individual fibers and myelin (arrows) PTAH X 1000.

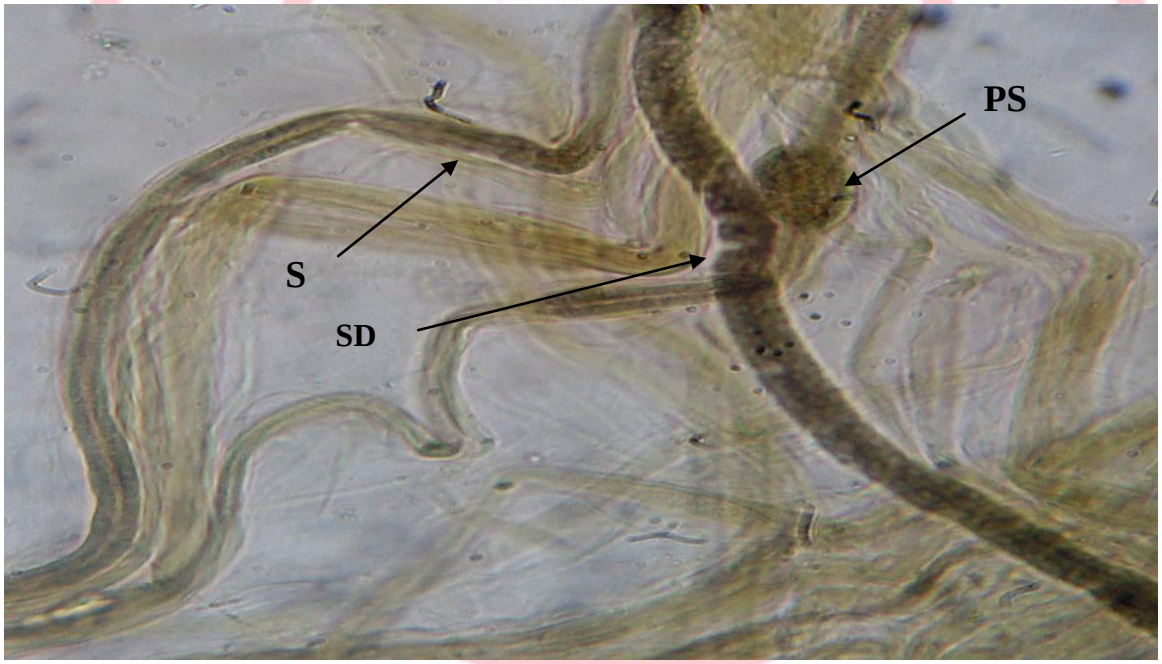

Figure (3): Teased fibers from TN of a diabetic patient showing short segments (S), paranodal swelling (PS) and segmental demyelination (SD). X 200.

The results of the histological sections for CPN and TN by PTAH stain

Diabetes showed a profound effect on both the CPN and TN in that; include a significant decrease in the number of individual fibers, a marked reduction in the diameter, and reduced thickness of myelin in both CPN and TN from diabetic individuals (Table 1, graph 1.figure 4-5) compared with controls (Figures 5, 6 and 7).

Table (1): The mean and SD of PTAH variables in the diabetic and control groups .

Group		N	Mean ± SD	95% Confidence interval of the difference	P value
CPNFN	Diabetic Control	30 30	24.13 ±2.21 59.83 ±2.61	-36.95 to -34.45	< 0.001
TNFN	Diabetic Control	30 30	19.37 ±1.47 40.57 ±1.01	-21.85 to -20.55	< 0.001
CPNFD	Diabetic Control	30 30	9.40 ±1.13 20.67 ±1.30	-11.90 to -10.64	< 0.001
TNFD	Diabetic Control	30 30	9.87 ±1.43 41.00 ±1.66	-31.93 to -30.33	< 0.001
CPNMT	Diabetic Control	30 30	1.07 ±0.25 7.00 ±0.64	-6.19 to -5.68	< 0.001
TNMT	Diabetic Control	30 30	1.23 ±0.43 4.47 ±0.78	-3.56 to -2.91	< 0.001

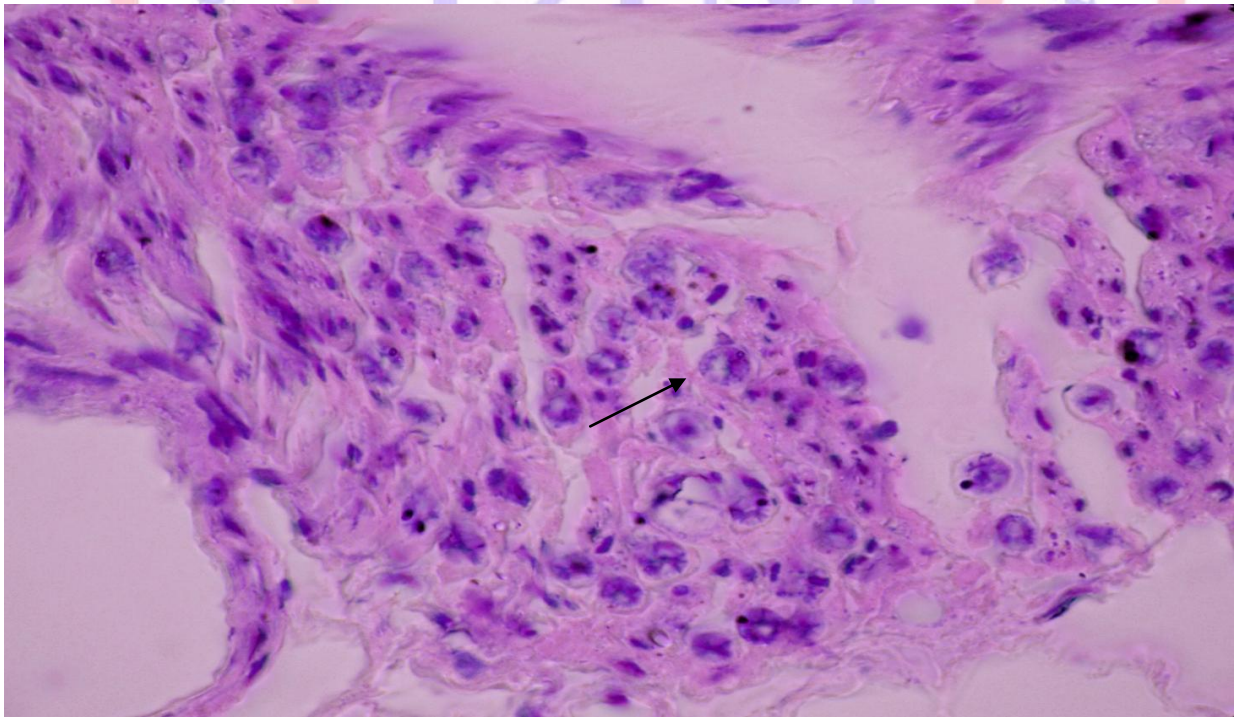

Figure (4): Cross section from TN from a diabetic person shows signs of demyelination (arrow) and loss of individual fibers X 1000.

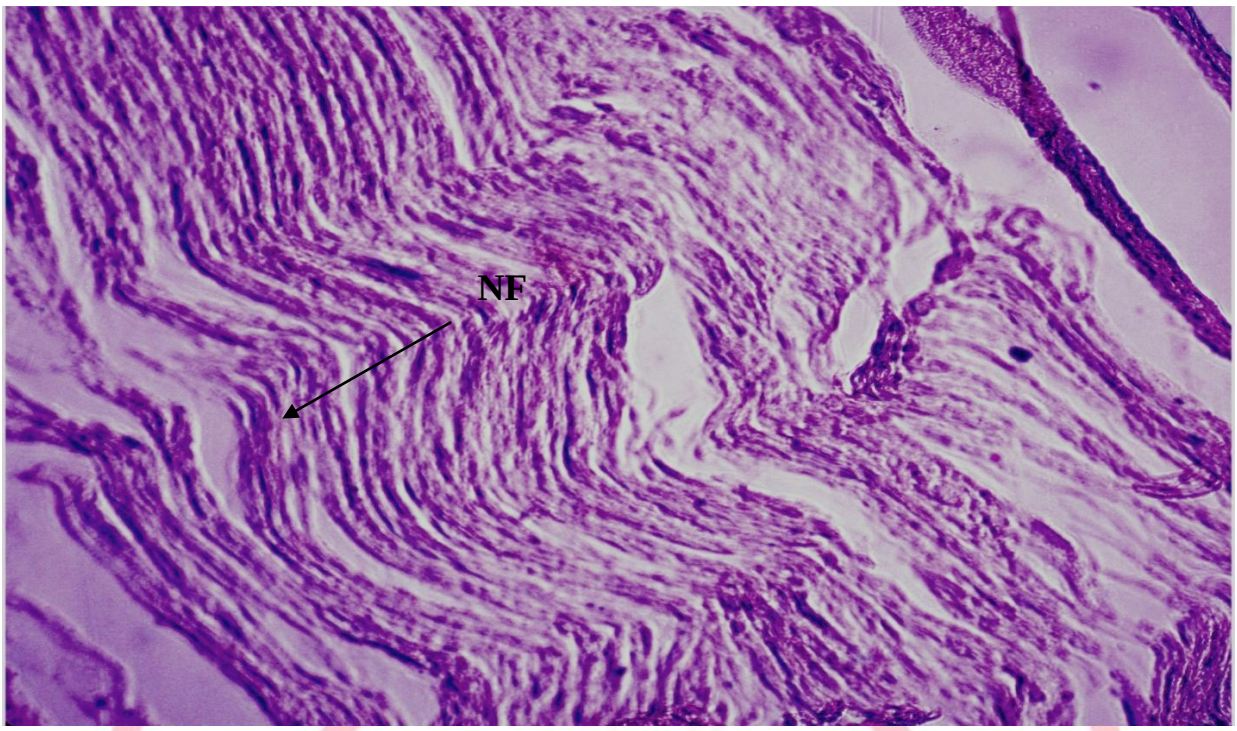

Figure (5): Longitudinal section from CPN of a diabetic person shows marked decrease in the number of individual fibers (NF). PTAH X 400.

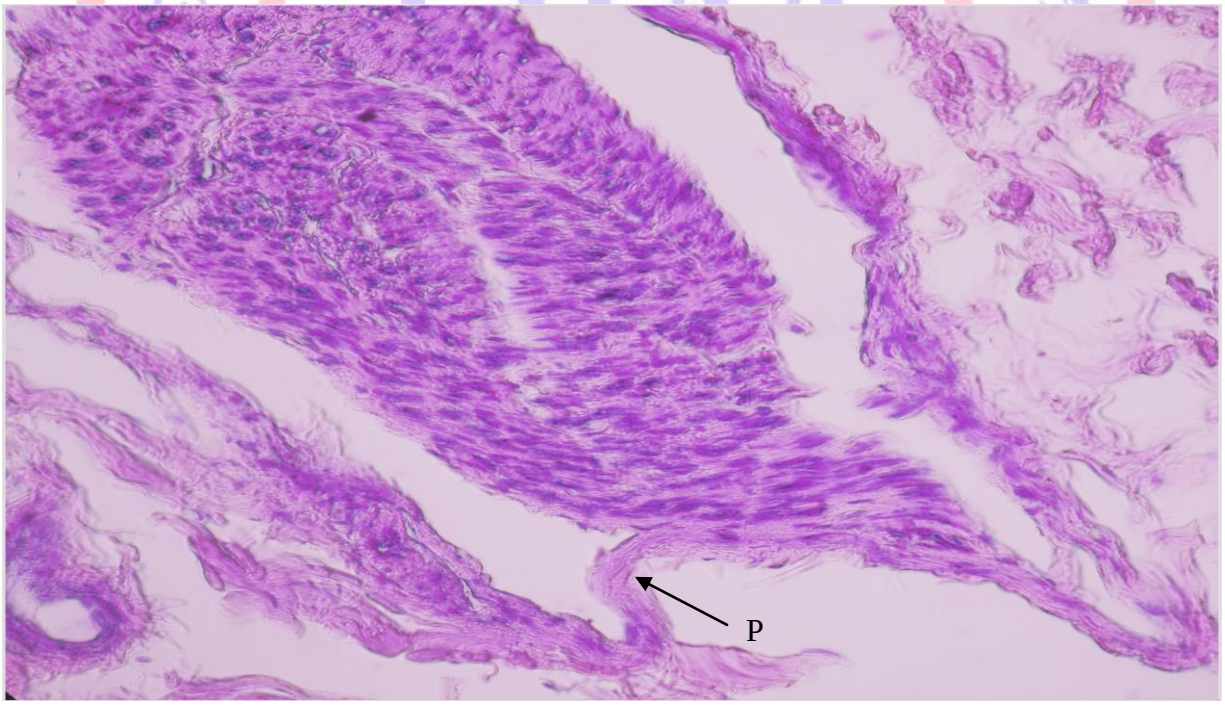

Figure (6): Section through CPN stained with PTAH, showing normal appearance X 400 (p= perineurium**).**

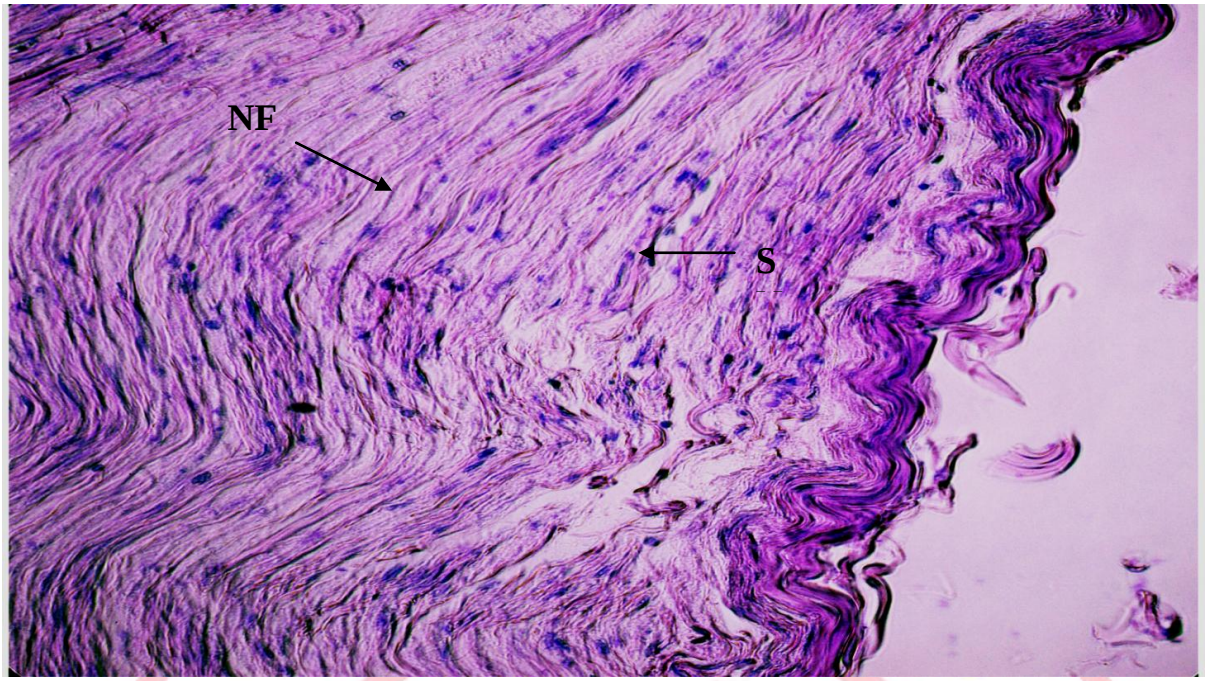

Figure (7): Longitudinal section from CPN of a normal control specimen, stained with PTAH showing normal architecture of the nerve fiber (SN=Shcwann cell nucleus, N=Nerve fiber) X 400.

Discussion

The segmental demyelination, paranodal swelling, shortening of intermodal segments, significant decrease in the number of the fibers, with a significant reduction in the nerve fiber diameter and the myelin thickness in the both nerves which obtained by this study in diabetic patients nerve samples , in agreement with that obtained by Sima and Brismar [11] and Sima et al. [12]. Hyperglycemia may lead to such changes because the normal metabolic function of Schwann cells are disturbed and stasis of blood and deposition of materials within blood vessels causing atherosclerosis of the wall of the blood vessel and thrombus formation. These changes consequently cause nerve ischaemia through a micro and macrovascular defect [13, and 14]. Another factor involved in the pathogenesis of diabetic neuropathy is the need for nerve regeneration after injury which is responsible for the appearance of short segments [15]. The complete length of the intermodal segment of the nerve fibers of un diabetic group was difficult to measured, maybe it is so long that cannot be followed by the microscope.

Observations of this study revealed that male gender are more vulnerable to develop diabetic peripheral neuropathy compared to women. The results are in agreement with, Kiziltan et al [16, and 17], while Manes et al [18] and Ugoya et al [19], showed no effect of gender on diabetic peripheral neuropathy. Furthermore, females have better endothelial function both in micro and macro circulations by the effect of the female endoneurial hormone progesterone and estrogen.

The possibility of the changes in common peroneal nerve is that there could be earlier damage of the small fibers by hyperglycemia [20]. Anatomically speaking, peroneal nerve is supplied by a few intraneural vessels of fine caliber so any obstruction of the fine caliber blood vessel leads to death of the nerve fibers [21 and 22], while the tibial

nerve receives abundant blood supply arising directly from the popliteal and posterior tibial artery that can support for oxygen supply.

The result of the present study showed that in diabetic individuals the number of fibers was moderately decreased in both CPN and TN, whereas in control group, there was moderate reduction in the number of the nerve fibers and myelin thickness in CPN. These results are in agreement with that obtained by Tesfaye et al [23], Resnick et al [24], Lindimuth et al [25], but in disagreement with that obtained by Tamir et al [26] who showed that age is not a risk factor for diabetic peripheral neuropathy.

Aging acts on apoptosis of the Schwann cells and leads to programmed cell death by an inflammatory process of aging which leads to decrease in the number of the myelin forming proteins, furthermore the reduction in the lumen of the vessels due to atherosclerotic changes in older subjects leading to ischemic degeneration of the nerve fibers consequently causing the decrease in the fiber number and the myelin thickness [27].

The duration of diabetes exerted a moderate inverse effect on the tibial nerve by decreasing the number of the fibers while no such effects were recorded on the common fibular nerve. This is in agreement with the results of the studies done by Riihimaa et al [28],Tamir et al [26], and Ugoya et al [19].

The long duration of exposure of the peripheral nerve fibers to the toxic effect of hyperglycemia leads to microangiopathy causing a decrease in the number of the fibers of the tibial nerve. This result is supported by Subramony et al (1982) cited by Jacobs [29].

In this study, the results of regression analysis of age, duration of diabetes and gender as independent variables on the various histological parameters of the common peroneal and tibial nerves showed that there was significant inverse effect of age on fiber number in both nerves when gender and duration of illness were constant. Gender related to myelin thickness of both nerves when age and duration of illness were constant, meanwhile myelin thickness of tibial nerve was closely related to duration of diabetes when age and gender were constant. This is in agreement with the results of the studies performed by Riihimaa et al [28], Resnick et al [24], Manes et al [18], Tamer et al [26], Martinez et al [31], Kiziltan et al [16], and Ugoya et al [19].

Recommendations

Performing imminohistochemistry and electron microscopy for Gene-mapping and the interaction of these genes with metabolic factors, are warranted.

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