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Effects of Omega-3 Fatty Acid on Diabetes Mellitus-Induced Histomorphological Changes in Peripheral Nerve

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Abstract

Objective: To study the protective effect of Omega 3 fatty acid co-administration in diabetes mellitus-induced histomorphological changes in peripheral nerve and nerve fibre diameter and fibrosis in perineurium in rats.

Methods: This was a Laboratory-based experimental animal study conducted in the Department of Anatomy, Army Medical College, Rawalpindi and National Institute of Health (NIH) Islamabad. Ninety male, healthy Sprague Dawley rats weighing 200-300gms were selected for the study and divided into three groups. Group A was the control group, group B was a diabetic group and group C was a diabetic group co-administered with omega-3 fatty acids orally for eight weeks. All rats were sacrificed and peripheral nerve samples were collected for gross and histological examination. Nerve samples were stained with Hematoxylin & Eosin and Masson Trichrome for microscopic examination. At the end of the experiment, nerves were examined for the diameter of the nerve and nerve fibres.

Results: The diameter of the nerve and nerve fibre was reduced in the diabetic group, but there was a statistically significant improvement in the omega-3 fatty acid group (group C) on the intergroup comparison. Fibrosis in the perineurium was also reduced in the omega-3 fatty acid group on intergroup comparison.

Conclusions: Diabetes mellitus causes histomorphological changes in the peripheral nerve and fibrosis in the perineurium which showed improvement in co-administration of Omega 3 fatty acid however on intergroup comparison between group A & C these protective effects were not statistically significant.

Keywords: Diabetes Mellitus, Diabetic Neuropathy, Fatty acids- Omega 3

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Introduction

Diabetes mellitus is a metabolic disorder which affects all the systems and organs of the body. It is the most common and fast-growing disease throughout the world.[1] The main feature of diabetes mellitus is persistently raised levels of blood glucose. The basic mechanism is that either there is either complete absence of insulin production in the type 1 diabetes mellitus (T1DM) early-onset autoimmune form, or a decreased level of insulin production or peripheral resistance which is type 2 diabetes mellitus (T2DM) or late-onset autoimmune form.[2] Almost all the systems and organs of the body are affected but the most frequent chronic complication of diabetes mellitus is diabetic neuropathies. Risk factors for prediabetes and diabetes mellitus include genetic atmosphere, a sedentary lifestyle, smoking alcoholism, dyslipidemia, decreased beta-cell sensitivity hyperinsulinemia, and improved glucagon activity.[3] Peripheral neuropathy associated with Diabetes mellitus affects up to 60% to 70% of patients. It results in progressive degeneration of the nerves. The most common early sign of diabetic neuropathy in humans is hyperalgesia due to damage to free nerve endings and the distal portions of sensory nerves.[4]
Factors which increase the risk of complications like distal polyneuropathy include long duration of diabetes mellitus, poor glycaemic control and raised basal metabolic index (BMI).[5]

Most extensive research is carried out on distal symmetric polyneuropathy (DSPN) and diabetic autonomic neuropathies, especially cardiovascular autonomic neuropathy (CAN). The most common type of chronic neuropathies is chronic DSPN, which is around 75% of the total diabetic neuropathies.[6] The most frequent clinical presentation is paresthesia and numbness because sensory nerves are more severely affected than motor. Common symptoms in the hands and feet are weakness, loss of balance, chronic pain and numbness. Both the myelinated and unmyelinated nerve fibres may be affected. There is numbness of the distal limbs, gait may also be affected and sometimes there is atrophy and weakness of muscles, whenever there is damage to the myelinated nerves. When the unmyelinated fibres are involved, there are painful neuropathy and autonomic symptoms.[7] This type of diabetic polyneuropathy results from the length-dependent degeneration of peripheral nerves and is not the axonal or demyelinating type. In peripheral nerves, penetration of glucose at high levels produces different pathologic metabolic reactions for example metabolic pathway (Polyol pathway) which converts glucose into sorbitol through the aldose reductase enzymes, high levels of intracellular sorbitol and fructose decreases active transport of several metabolites, myoinositol is one of them.[8] DSPN can develop in prediabetes as well so early diagnosis and prevention is important. The use of dietary long-chain omega-3 polyunsaturated fatty acids has many benefits in general including prevention of cardiac arrhythmia, lowering of triglyceride levels, amelioration of inflammation, and prevention of neuropathy.[9]

The objective of the study was to study the protective effect of Omega-3 fatty acid co-administration in Streptozotocin-induced diabetic peripheral neuropathy. Omega-3 fatty acids oral supplementation has proven to promote nerve regeneration in diabetes mellitus type 1 patients.[10]

Materials And Methods

It was a lab-based experimental study carried out in Army medical college. Rawalpindi and National Institute of Health Islamabad. Ninety Sprague Dawley rats were taken and divided into three groups. Each group comprised thirty rats. Group A was the control group, group B was a diabetic group and group C was diabetic with co-administration of Omega-3 fatty acid supplementation.

Diabetes was induced in experimental groups by injection of streptozotocin (STZ). A freshly prepared single intra-peritoneal injection of STZ was given in overnight fasted animals at a dose of 55 mg/kg body weight. The animals were given a 5% glucose solution to drink overnight. The rats with hyperglycemia, blood glucose ≥ 250 mg/dl, on the 4th day after STZ injection confirmed by blood glucose estimation from the tail vein by using doing tail vein puncture were selected. [11]

Animals of groups A, B and C were on a standard lab diet for 8 weeks. In group C animals at the time of induction of diabetes, the rats were Co-administered with Omega-3 Fatty acid 12.5mg per rat orally. [12] It was administered via oral gavage tube for eight weeks.

The animals were sacrificed after two months of the experimental period. 13 At the end of 8 weeks, the animals were euthanized by placing ether-soaked cotton in the jar. The rats were placed on a dissection board. The previous study shows the appearance of peripheral neuropathies after a week's experimental period. The tibial nerve 1-1.5cm section was taken from the mid portion. The diameter of the tibial nerve in millimetres(mm) was measured using a vernier calliper. Tissue after excision was put in 10% neutral buffered formalin for fixation. Nerve transverse and longitudinal sections were obtained for processing. 5µm thick sections were cut. Sections were stained with Hematoxylin and Eosin for routine histological study of Masson's trichrome for connective tissue deposition. On microscopic examination, epineurium and perineurium stained dark eosinophilic as compared to the nerve fibres which stained light pink on H&E staining. Adipose cells and myelin are unstained on H&E staining. The Myelin of the myelinated nerves was unstained so it appeared like a clear hallow around the light pink stained nerve.[14] For the measurement of nerve fiber diameter four consecutive high power fields per section were selected and three random nerve fiber diameters were measured in each fascicle in cross section. The diameter of nerve fibres (axons) which were rounded pink coloured (eosinophilic) was measured in two axes and then the average was calculated. Fibrosis in the perineurium was observed on Masson Trichrome staining on 40-X magnification and scored as 0 when absent (Scanty connective tissue of perineurium) and 1 when present (Widening of perineurium with comparatively increased amount of connective tissue). 15 It was in the form of the bluish stained area in between the nerve bundles.

Micrometry was done to quantitatively measure the nerve fibre diameter microscopically. It was performed in four consecutive high-power fields per section. Three nerve fibre diameters were measured in each nerve randomly. Nerve fibres (rounded eosinophilic) diameters were measured in two axes and then the average was calculated. Fibrosis in the perineurium was also observed on Masson Trichrome staining on 40-X magnification and scored as 0 when absent (Scanty connective tissue of perineurium) and 1 when present (Widening of perineurial spaces with comparatively increased amount of connective tissue).[15] It was in the form of bluish stained area in between the nerve bundles.

Results

In control group A the mean diameter of the nerve was 2.012 ±0.15mm (Table) in experimental group B1.266 ±0.25mm (Table) and group C 1.450 ±0.388mm (Table). On intergroup comparison p-value for A vs B was 0.000*, A vs C 0.000*& B vs C 0.005 the difference was statistically significant among all groups. The mean diameter of nerve fibre in control group A was 2.01 ±0.159µm (Table) in group B 1.26 ±0.253µm (Table) and group C was 1.45 ±0.388µm (Table). On intergroup comparison p-value for A vs B was 0.000*, A vs C 0.000* & B vs C 0.005 it was statistically significant between all gIn control group A fibrosis in perineurium was absent in all animals (Table). In experimental group B, it was present in all animals (Table). In group C it was present in 23 animals (Table). On intergroup comparison p-value for Avs B was 0.000*, A vs C 0.000* & B vs C 0.000*. It was statistically significant among all groups (Table).

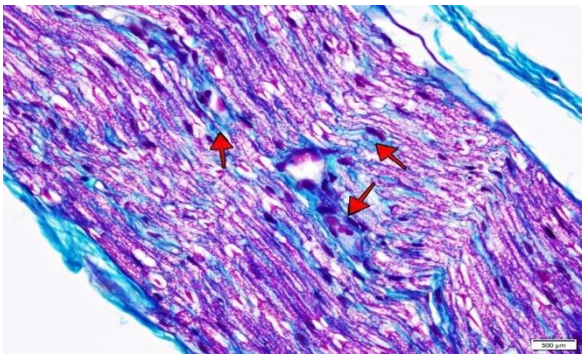

Figure 2: A photomicrograph of a cross-section of the tibial nerve of an animal (Experimental group B) showing fibrosis (Red arrows), Masson Trichrome, 40-

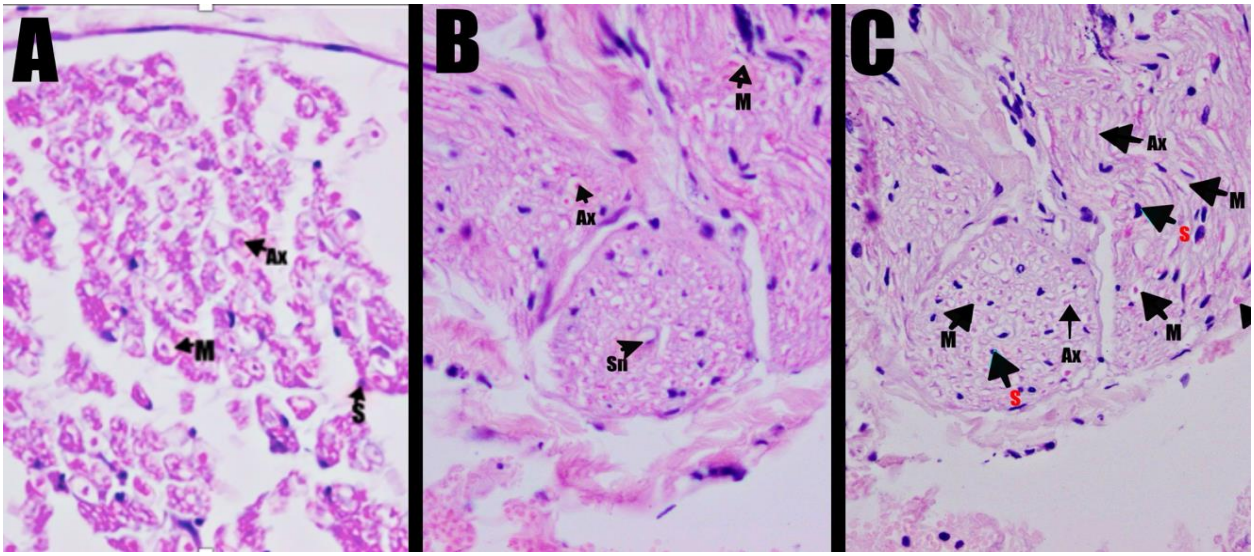

Figure 1: A photomicrograph of a cross-section of the tibial nerve of an animal (Group A, B & C) showing Axons (Ax), Schwann cells (S) & Myelin (M). H&E, 40-X

Table 1: Comparison of the diameter of nerve & diameter of nerve fibre in control group A, experimental groups B and group C, Comparison of fibrosis in the perineurium

Parameters	Groups	Mean ± SD	Statistical Significance		
			Group A vs B	Group A vs C	Group B vs C
Diameter of nerve (mm)	A	2.012 ±0.159	0.000*	0.000*	0.005
	B	1.266 ±0.253			
	C	1.450 ±0.388			
Nerve fiber diameter(µm)	A	2.01 ±0.159	0.000*	0.000*	0.005
	B	1.26 ±0.253			
	C	1.57 ±0.388			
Fibrosis	**A**	**B**	**C**	0.000*	0.000*	0.000*
Absent	30 (100%)	Zero (0.0%)	7 (23.3%)			
Present	Zero (0.0%)	30 (100%)	23 (76.7%)			

**p-value < 0.001 is statistically significant*

Discussion

Diabetes mellitus leads to neuropathy and in this study, the ameliorative effects of Omega-3 fatty acid on diabetic neuropathy were evaluated through the parameters of nerve and nerve fiber diameter and fibrosis in perineurium. Diabetic neuropathy evaluation was done through various parameters. The diameter of the tibial nerve was measured and compared grossly. It was reduced in the diabetic group however omega-3 fatty acid had ameliorative effects and the diameter of the nerve in this group was less reduced (Table). The mechanism for the development of diabetic neuropathy is the deposition of various metabolites in the peripheral nerves resulting in oxidative stress which can also be due to hyperglycemia which causes impairment of vascular supply. Chronic hyperglycemia has damaging effects on the blood-nerve barrier. When proteins like albumin and immunoglobulins enter and accumulate in nerves by penetrating the endoneurium for a long period, there is a disturbance of electrolyte regulation in peripheral nerves, and this leads to the development of oedema, resulting in the ischemia of the nerve fascicles.[16] There is a decreased level of nerve growth factors and accumulation of various inflammatory mediators responsible for the pathogenesis of neuropathy.[17] In this type of peripheral neuropathy there is axonal degeneration caused by a metabolic disorder affecting peripheral nerve tissue due to hyperglycemia and by disruption of the homeostatic mechanism of peripheral nerves affecting the blood-nerve barrier and Schwann cells.[16] Nerve fibre diameter was significantly reduced in the diabetic group but omega-3 fatty acid co-administration has significantly reduced the effects of diabetes (Table). Omega-3 fatty acids (PUFA) are essential fatty acids that have important roles in different physiological processes, like neural health, integrity of membrane structure, anti-inflammatory processes, and lipid metabolism. PUFA-derived mediators like resolvins, neuroprotectin, and maresins which also favour nerve regeneration, show that omega-3 fatty acid is a potential treatment option in distal symmetric polyneuropathy (DSP). Studies in PUFA-treated animals of DSP have shown benefits in function and improvement in pathological conditions. Recent research shows that omega-3 fatty acid has a beneficial effect on the regeneration of small nerve fibres with an increase in the length of a corneal nerve fibre (small nerve fibre diameter).[18] It is by a previous study indicated that the nerve fibres of peripheral nerves in STZ-induced diabetes rats were loosely arranged and the number and the diameter of nerve fibres were reduced also there was shrinkage of axons in the cross section.[19] There was no fibrosis in the perineurium of control group when seen on Masson Trichrome staining with very little bluish stained areas visible while in diabetic group there was fibrosis in the perineurium. In the omega-3 fatty acid group, there was fibrosis in perineurium but lesser than untreated diabetic group (Table). It is a previous study showing the presence of fibrosis with atrophic nerve fibres. The pathogenesis for fibrosis in diabetic neuropathy includes microvascular damage, metabolic damage and immune response. Diabetes causes impairment of acetylcholine-induced vasodilatation as a result there is a reduction in the blood supply to the nerves. The blood-nerve barrier is damaged which causes edema in the endoneurium as a result fibrotic tissue replaces the atrophied nerve tissue. [20] Another study shows there was thickening and perineurial fibrosis in the untreated diabetic group than control group. [21] In the chronic phase, mechanical and ischemic damage occurs so there is damage to capillaries as well as perineurium (blood nerve barrier). Intra-fascicular edema is slowly reabsorbed and then it is replaced by interstitial fibrosis. Fibrosis is the Excessive deposition of collagen. Many degenerative changes develop progressively within different sections of the peripheral nerve. These include damage and disruption of axons, degradation of myelin sheaths with the formation of globules and debris which are slowly cleaned by macrophages and replacement of the Schwann cells by fibroblasts producing collagen fibres. [22]

Conclusions

Diabetes mellitus causes peripheral nerve damage by causing Peripheral nerve fibre atrophy and fibrosis in the perineurium. These two parameters were reduced by Omega-3 fatty acid administration. Diabetes mellitus causes histomorphological changes in peripheral nerves which showed improvement in co-administration of Omega-3 fatty acid in some histological parameters but on intergroup comparison between groups A & C this was not statistically significant.

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S.H - Critical Review

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