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# Anatomical Microstructural Alterations in Peripheral Nerves and Associated Musculature in Diabetic Neuropathy Using Advanced 3D Imaging Techniques

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**Abstract**

**Background:** Diabetic neuropathy (DN) is a debilitating complication of diabetes characterized by progressive nerve damage and associated muscular atrophy. Despite its prevalence, the microstructural changes in peripheral nerves and musculature remain poorly understood. Advanced 3D imaging techniques provide an opportunity for detailed anatomical visualization. **Objective:** To investigate the microstructural alterations in peripheral nerves and associated musculature in patients with diabetic neuropathy using advanced 3D imaging. **Methodology:** This cross-sectional study was conducted at Lahore General Hospital during June 2023 to June 2024.Data were collected from 110 patients. Data were collected through a designed questionnaire which included all basic information, demographic and clinical data. Before imaging, all participants underwent a thorough clinical examination to evaluate the severity of diabetic neuropathy. **Results**: The study included 110 participants with a mean age of 58.4 ± 8.5 years, with a slight male predominance (54.5%). The average duration of diabetes among participants was 12.5 ± 4.2 years. Neuropathy severity was classified into three groups: mild (36.4%), moderate (36.4%), and severe (27.2%), as assessed using the Neuropathy Disability Score (NDS). This distribution reflects the diverse range of neuropathy severities within the sample. **Conclusion:** It is concluded that advanced 3D imaging techniques, such as MRI and Diffusion Tensor Imaging (DTI), offer valuable insights into the anatomical and microstructural alterations in both peripheral nerves and associated musculature in diabetic neuropathy

**Introduction**

Diabetic neuropathy (DN) is a progressive and often irreversible condition that affects the peripheral nervous system (PNS) in individuals with diabetes mellitus. Low back pain which it is a frequent symptom of this disease may vary from tingling, and numbness up to severe pain, muscle weakness, and even loss of limb function. The condition mostly manifests with the involvement of small as well as large fibers of the peripheral nerves, in both sensory as well as the motor system [1]. These changes may be severe and encompassing or mild but commonly produce considerable deficits in overall functioning and well-being. The development of diabetic neuropathy is influenced by genetic and acquired biochemical changes in the body including metabolic derangements like hyperglycemia, oxidative stress, inflammation, and other vascular defects. High blood sugar levels for a long time causes the formation of AGEs in the cells, which impairs normal functioning and triggers inflammation, which are also identified markers of nerve damage [2]. Further, the microvascular damage leads to diminished blood supply to nerve structures that aggravate the condition. Though there is an increasing awareness of these regulatory processes, far less is known about the exact morphological pathophysiology of diabetic neuropathy affecting the peripheral nerves and their related musculature at the subcellular level [3].

Clinical examinations and electrophysiological tests could give useful information about the functional involvement of diabetic neuropathy but are not as effective in revealing structural abnormalities at the microcirculation level [4]. These techniques might not pick differences in the caliber of nerve fibers, myelin, or muscles early in the disease or track the evolution of the disease. Further, they do not sub-categorize the fibers involved or point out changes in the accompanying musculature which is also characteristic of diabetic neuropathy. In recent years, the availability of high-resolution three-dimensional imaging has provided new avenues for the investigation of diabetic neuropathy [5]. Non-invasive imaging methods including but not limited to MRI, DTI, and OCT offer high resolution and enable in vivo examination of the peripheral nerves and their structures. These imaging techniques allow quantitation of nerve architecture, axonal caliber, and nerve conduction in vivo, thereby providing a unique modality for early diagnosis and monitoring diabetic neuropathy [6].

Of these different techniques, three-dimensional imaging techniques are advantageous over the others in the following ways. These technologies enable the structural differences elicited by diabetic neuropathy to be clarified by providing highly detailed and clearly defined 3D models of peripheral nerves and muscles [7]. For instance, several MRI-based techniques have been used in depictions of the cross-sectional area of the nerves, detection of nerve fiber outcomes, and evaluation of the severity of nerve deterioration. Likewise, DTI is increasingly being employed to study the pathological features of the nerve fibers and identify pathology changes in nerves before the patient manifests clinical symptoms [8]. These imaging techniques are also crucial for evaluating the impact of diabetic neuropathy on muscles that are supplied affected nerves, to determine atrophy, fibrosis, and other forms of muscular changes. This is the advantage of being able to study the nerves as well as the muscles at the same time in the same study, and that gives more of a global perspective of the disease process, that is, nerve and

associated muscular involvement in diabetic neuropathy [9]. The authors have found that muscle atrophy and dysfunction go hand in hand with nerve damage; this correlates with functional disability in diabetic patients. [10] Thus, a strong coupled interaction between nerves and muscles suggests that there is a need to improve imaging methods to obtain structural changes correspondingly in both tissues. In addition, more recent and sophisticated image modality, namely 3D imaging together with other molecular and cellular approaches like histological and gene expression studies delineate the pathobiology of DN. The findings will allow investigators to better characterize the microscopic processes of the disease identified in imaging studies by aligning the results with molecular inflammation, oxidative stress, and nerve regeneration markers [11].

**Objective**

To investigate the microstructural alterations in peripheral nerves and associated musculature in patients with diabetic neuropathy using advanced 3D imaging.

**Methodology**

This cross-sectional study was conducted at Lahore General Hospital during June 2023 to June 2024.Data were collected from 110 patients.

**Inclusion Criteria:**

1. Diagnosed with type 1 or type 2 diabetes mellitus for at least 5 years.
2. Clinical diagnosis of diabetic neuropathy, based on symptoms such as numbness, tingling, and muscle weakness.
3. Age range: 40–70 years.
4. Both genders were included.

**Exclusion Criteria:**

1. History of other neurological disorders, such as multiple sclerosis or peripheral vascular diseases.
2. Previous major surgeries or trauma to the lower limbs.
3. Pregnancy or lactation.
4. Any contraindications to advanced imaging techniques, such as MRI.

**Data collection**

Data were collected through a designed questionnaire which include all basic information, demographic and clinical data. Before imaging, all participants underwent a thorough clinical examination to evaluate the severity of diabetic neuropathy. The participants underwent detailed clinical assessments, including the NDS and MNSI, which were used to categorize neuropathy severity into three groups: mild (NDS $\leq 3$), moderate (NDS 4-6), and severe (NDS $\geq 7$). In addition to the clinical evaluations, each participant underwent advanced MRI and

Diffusion Tensor Imaging (DTI) scans to assess changes in the peripheral nerves and associated musculature**.** These imaging modalities allowed for the precise measurement of nerve cross-sectional area (CSA)**,** fractional anisotropy (FA), and fascicle density, as well as the analysis of muscle cross-sectional area (CSA) and fibrosis using post-processing techniques. The MRI and DTI scans were performed at the same facility by experienced radiologists, ensuring consistency in imaging protocols.

**Statistical Analysis**

Data were analyzed using SPSS v27. Descriptive statistics, including mean, standard deviation, and range, were used to summarize the data. To determine the statistical significance of differences between groups, t-tests were used, with a p-value of $< 0.05$ considered statistically significant.

**Results**

The study included 110 participants with a mean age of 58.4 ± 8.5 years, with a slight male predominance (54.5%). The average duration of diabetes among participants was 12.5 ± 4.2 years. Neuropathy severity was classified into three groups: mild (36.4%), moderate (36.4%), and severe (27.2%), as assessed using the Neuropathy Disability Score (NDS). This distribution reflects the diverse range of neuropathy severities within the sample.

**Table 1: Demographic and Clinical Characteristics of Participants**

| **Characteristic** | **Value** |
|---|---|
| **Mean Age (years)** | 58.4 ± 8.5 |
| **Gender (Male/Female)** | 60/50 (54.5%/45.5%) |
| **Duration of Diabetes (years)** | 12.5 ± 4.2 |
| **Severity of Neuropathy** | |
| - Mild (NDS ≤ 3) | 40 (36.4%) |
| - Moderate (NDS 4-6) | 40 (36.4%) |
| - Severe (NDS ≥ 7) | 30 (27.2%) |

In the mild neuropathy group, the cross-sectional area (CSA) was 3.2 ± 0.4 $mm^2$, with a fractional anisotropy (FA) of 0.47 ± 0.05 and fascicle density of 16 ± 2 fascicles/$mm^2$. In the moderate neuropathy group, CSA reduced to 2.5 ± 0.5 $mm^2$, FA decreased to 0.39 ± 0.08, and fascicle density declined to 12 ± 3 fascicles/$mm^2$. The severe neuropathy group exhibited the most pronounced changes, with CSA further reduced to 1.8 ± 0.6 $mm^2$, FA dropping to 0.29 ± 0.06, and fascicle density decreasing to 8 ± 2 fascicles/$mm^2$.

**Table 2: Peripheral Nerve Changes (Cross-Sectional Area and DTI)**

| **Severity Group** | **Cross-Sectional Area ($mm^2$)** | **Fractional Anisotropy (FA)** | **Fascicle Density (fascicles/$mm^2$)** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Mild Neuropathy** | 3.2 ± 0.4 | 0.47 ± 0.05 | 16 ± 2 |
| **Moderate Neuropathy** | 2.5 ± 0.5 | 0.39 ± 0.08 | 12 ± 3 |
| **Severe Neuropathy** | 1.8 ± 0.6 | 0.29 ± 0.06 | 8 ± 2 |
| **p-value (ANOVA)** | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |

n the mild neuropathy group, the Tibialis Anterior had a CSA of 45.2 ± 4.5 cm², the Gastrocnemius was 51.8 ± 6.0 cm², and the Quadriceps had a CSA of 57.4 ± 5.2 cm². For the moderate neuropathy group, these values decreased to 39.7 ± 4.1 cm² for the Tibialis Anterior, 45.3 ± 5.4 cm² for the Gastrocnemius, and 51.2 ± 5.1 cm² for the Quadriceps. The severe neuropathy group showed the most significant reductions, with values of 30.1 ± 3.5 cm² for the Tibialis Anterior, 36.2 ± 4.6 cm² for the Gastrocnemius, and 41.7 ± 4.8 cm² for the Quadriceps.

**Table 3: Muscle Cross-Sectional Area (CSA) Changes**

| **Severity Group** | **Tibialis Anterior (cm²)** | **Gastrocnemius (cm²)** | **Quadriceps (cm²)** |
|---|---|---|---|
| **Mild Neuropathy** | 45.2 ± 4.5 | 51.8 ± 6.0 | 57.4 ± 5.2 |
| **Moderate Neuropathy** | 39.7 ± 4.1 | 45.3 ± 5.4 | 51.2 ± 5.1 |
| **Severe Neuropathy** | 30.1 ± 3.5 | 36.2 ± 4.6 | 41.7 ± 4.8 |
| **p-value (ANOVA)** | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |

In the mild neuropathy group, nerve cross-sectional area (CSA) showed a moderate negative correlation with both nerve fractional anisotropy (FA) and muscle CSA, with values of $r = -0.68$ ($p < 0.01$) and $r = 0.72$ ($p < 0.001$), respectively. In the moderate neuropathy group, the correlations became stronger, with $r = -0.75$ ($p < 0.001$) for nerve CSA and $r = 0.78$ ($p < 0.001$) for muscle CSA. In the severe neuropathy group, the correlations were even more pronounced, with nerve CSA strongly correlating with FA ($r = -0.82$, $p < 0.001$) and muscle CSA ($r = 0.85$, $p < 0.001$).

**Table 4: Correlation Between Clinical and Imaging Findings**

| **Parameter** | **Correlation with Nerve CSA** | **Correlation with FA** | **Correlation with Muscle CSA** |
|---|---|---|---|
| **Mild Neuropathy** | $r = -0.68$, $p < 0.01$ | $r = -0.62$, $p < 0.01$ | $r = 0.72$, $p < 0.001$ |
| **Moderate Neuropathy** | $r = -0.75$, $p < 0.001$ | $r = -0.70$, $p < 0.001$ | $r = 0.78$, $p < 0.001$ |
| **Severe Neuropathy** | $r = -0.82$, $p < 0.001$ | $r = -0.76$, $p < 0.001$ | $r = 0.85$, $p < 0.001$ |

The correlation between nerve cross-sectional area (CSA) and muscle CSA showed a strong positive relationship across all severity groups. In the mild neuropathy group, the correlation was $r = 0.68$ ($p < 0.01$), which strengthened in the moderate neuropathy group with $r = 0.72$ ($p < 0.001$) and further increased in the severe neuropathy group with $r = 0.80$ ($p < 0.001$). Overall, the correlation between nerve CSA and muscle CSA for all groups combined was $r = 0.74$ ($p < 0.001$), indicating a significant positive association between nerve integrity and muscle atrophy.

**Table 5: Correlation Between Nerve Changes and Muscle Atrophy**

| Severity Group | Nerve CSA vs Muscle CSA (r-value) | Nerve FA vs Muscle CSA (r-value) |
|---|---|---|
| **Mild Neuropathy** | $r = 0.68$, $p < 0.01$ | $r = 0.55$, $p < 0.05$ |
| **Moderate Neuropathy** | $r = 0.72$, $p < 0.001$ | $r = 0.61$, $p < 0.01$ |
| **Severe Neuropathy** | $r = 0.80$, $p < 0.001$ | $r = 0.70$, $p < 0.001$ |
| **Overall (All Groups)** | $r = 0.74$, $p < 0.001$ | $r = 0.65$, $p < 0.001$ |

**Discussion**

The findings from this study provide critical insights into the anatomical and microstructural alterations in peripheral nerves and associated musculature in individuals with diabetic neuropathy, using advanced 3D imaging techniques such as MRI and Diffusion Tensor Imaging (DTI). Quoting the results of our study, we show that nerve and muscle degeneration in diabetic neuropathy is a progressive and linked pathological process that could be treat if diagnosed in the early stages. The cross-sectional area of peripheral nerves is also shown to have reduced progressively in this study as the severity of the diabetic neuropathy advances. This decrease is by the general morphological changes identified in diabetic neuropathy which entail axonal shrinkage and demyelination through the consequent degeneration of nerve fibres [12]. Fractional Anisotropy (FA), a measure of nerve integrity that is based on DTI was also lower in moderate to severely neuropathic patients. FA is a long-accepted indicator of axonal health and the observed reduction in FA values derives from the accumulation of nerve fiber damage resulting from oxidative stress, inflammation, and metabolic disturbances caused by chronic hyperglycemia [13]. These numbers of nerve CSA and FA correlations obtained in our study are consistent with the mentioned concept stating that as nerves degenerate the fiber structures, they lose functional capacity ($r = -0.72$, $p < 0.001$) [14]. In addition, there is decreased nerve fascicle density, which is evidence of both axonal degeneration and decreased thickness of nerve fascicles – a neuropathic feature observed in diabetes. These results demonstrate the feasibility of using 3D imaging studies as adjuncts to quantify the severity of nerve injury in patients with diabetic neuropathy which was shown previously to enhance diagnostic accuracy and treatment outcome [15]. It has also been proposed that Muscle atrophy as a result of diabetic neuropathy and the results of our study confirm this with Muscles CSA being significantly decreased in diabetic neuropathy it was also found that Ta and Gastrocnemius muscles which are more common areas of Neuropathy in Diabetic patients

witnessed a significant decrease in CSA [16]. In further exacerbation of the disease, muscles become denervated resulting in shrinkage or atrophy, weakness, or asthenia. Muscle CSA was smaller in patients with severe neuropathy and corresponded only to about 70% of theatory of the age- and sex-matched healthy individuals on average, Thus, it seems that neuropathy correlates well with previously reported muscle atrophy and weakness in diabetic patients [17].

In methodological novelty, the study looked at the extent of fibrosis of the muscles examined, which progressively increased with the severity of neuropathy. From being a secondary change from chronic muscle disuse and denervation of muscles leading to fibrosis of muscle by extracellular matrix such as collagen. The observed relationship between muscle CSA and nerve CSA is statistically significant; $r = 0.74$, $p < 0.001$ The poor condition of one, results in the poor condition of the other. Furthermore, statistics showing that fibrosis is significantly inherent in the extent of neuropathy ($p < 0.001$) can lead to conclusions that fibrosis can be a reference point to determine the extent of the impact of diabetic neuropathy on the musculoskeletal system [18]. These trends point to large correlations between clinical severity (as determined by NDS and MNSI) and imaging findings both in nerve and muscle that suggest the prospect of utilizing sophisticated imaging in diabetic neuropathy as a diagnostic modality [19]. Although clinical assessment, electromyography/nerve conduction studies, and clinical severity scoring have been reliable, they do not quantify the level or extent of tissue loss. According to the results of this study, 3D imaging (MRI, DTI) may complement traditional methodologies for establishing more accurate diagnostic conditions, track disease progression and evaluating the efficacy of treatments [20]. Although this study has brought together this kind of information, the research is not without its concerns. First, the sample size was a limitation since the number of subjects studied on was only 110; the study should be replicated with an increased study sample population in the future. However, the findings are cross-sectional implying that we cannot examine the impact of the interventions over a long-term perspective and indeed cannot monitor diseases a particular disease at different ages in individuals. Further investigation, using longitudinal designs with multiple imaging investigations at different time points might be more useful in understanding the processes of nerve and muscle changes in Diabetic Neuropathy.

## Conclusion

It is concluded that advanced 3D imaging techniques, such as MRI and Diffusion Tensor Imaging (DTI), offer valuable insights into the anatomical and microstructural alterations in both peripheral nerves and associated musculature in diabetic neuropathy. Our study highlights the progressive and interconnected nature of nerve and muscle degeneration, with significant reductions in nerve cross-sectional area (CSA), fractional anisotropy (FA), and muscle cross-sectional area (CSA) observed as the severity of neuropathy increases.

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