Increased Density of Axonal Spheroids in the Nucleus Gracilis of the Lower Brainstem in Diabetic Versus Non-Diabetic Patients

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Abstract

The presence of axonal spheroids is unusual in the absence of a clinical history of CNS injury. Nevertheless, increased numbers of axonal spheroids in the lower brainstem have been consistently observed in autopsied diabetic patients. A prospective comprehensive investigation of the density, size, and distribution of axonal spheroids in the brainstem and spinal cord was undertaken in 22 patients and correlated with comorbidities, age, and gender. In most cases, an increased density of axonal spheroids was identified within the nucleus gracilis of the lower brainstem. Moreover, the highest densities ($p = 0.013$) and circumferences ($p = 0.002$) of axonal spheroids were present in the lower brainstem of diabetics when compared to non-diabetics. Whereas the pathology of peripheral neuropathy in diabetics is well described, this study is the first demonstration of specific CNS pathology in diabetic patients.

Introduction

Length dependent peripheral neuropathy is common in chronic diabetes, however, concomitant axonal injury in the CNS is less well described (1,2). The spinal cords of diabetics commonly show significant degeneration of the spinal tracts especially the posterior columns (fasciculi gracilis and cuneatus)(1). The nucleus gracilis receives information from the ascending fibers of the fasciculus gracilis that includes fine discriminating touch, pressure, vibratory sense and proprioception from the lower portion of the body (3). The presence of axonal spheroids is indicative of axonal injury. In a blinded prospective study, we examined the brainstem and spinal cords of 22 autopsied diabetic and non-diabetic patients for the presence and distribution of axonal spheroids. Our data indicates there is a generalized increase in density of axonal spheroids localized to the nucleus gracilis in all patients, but the average density and axonal spheroid circumference is significantly increased in diabetic patients.

Methods

The brainstem and spinal cords from 22 patients with and without diabetes were collected at autopsy, formalin fixed and entirely embedded in paraffin at 0.5 cm intervals. Sections were stained with hematoxylin and eosin, scanned with Aperio Scanscope XT scanner and then analyzed using ImageScope software. Each section was carefully examined for axonal spheroids larger than 50 µm in circumference and scored. A 50 µm threshold was chosen since axonal spheroids smaller than 50 µm in circumference could not be reliably distinguished from normal structures and tissue artifacts. The axonal spheroids were further subcategorized based upon the presence or absence of eosinophilic inclusions hypothesized to represent a spectrum of degenerative change. All candidate axonal spheroids were reviewed by two observers (TM and LK) for consensus categorization without prior knowledge of the patient's clinical history. The data was statistically analyzed utilizing Microsoft Excel and correlated with age, gender, diabetic history, atherosclerotic heart disease, and known central nervous system infarcts by volume in mm^3. To determine statistical significance ($p < 0.05$) of two variables, the Levine's test was performed for variance followed by the Student's T-Test. For comparing multiple variables, one-way ANOVA was used.

Results

Table 1. Patient Demographics. Distribution of patients by gender and comorbidities including diabetes, atherosclerotic cardiovascular disease (ASCVD), and known central nervous system (CNS) infarcts.

		Range	Median
Age	Male	31-92	65
	Female	55-81	66
	Overall	31-92	65
		N	Percentage (%)
	Total	22	--
Gender	Male	11	50.0
	Female	11	50.0
Clinical History	Diabetes	9	40.9
	ASCVD	9	40.9
	CNS Infarcts	9	40.9

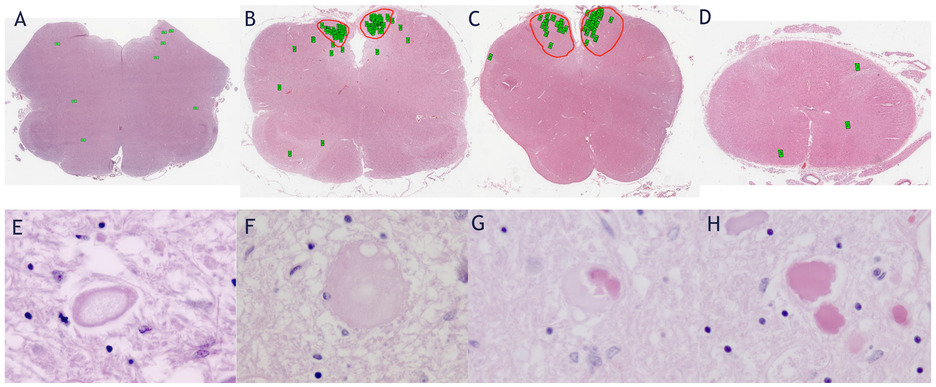

Figure 1. Representative Sections of Brainstem and Spinal Cord with Distribution of Axonal Spheroids. (A) Upper medulla. (B, C) Lower Medulla with Nucleus Gracilis outlined in red. (D) Spinal Cord. Axonal Spheroids are marked in green and classified according to presence of degenerative changes (1-3). Axonal spheroids were subclassified into three groups based upon morphology (E-H): 1(E) contains areas hypodense to background, 2(F) staining isodense with background, 3(G, H) presence of hypereosinophilic (hyperdense) inclusions. All sections were stained with hematoxylin and eosin. Original magnification for E-H is 1000X.

Table 2. Average Axonal Spheroid (AAS) Density per Patient (N) by Location.

Location	AAS Density per Section/N	Standard Deviation	P-Value
Upper Medulla	3.6	3.2	
Lower Medulla	41.1	24.4	$4.4 \cdot 10^{-9}$
Spinal Cord	7.9	6.5	

Table 3. Average Axonal Spheroids (AAS) and Average Degenerating Axonal Spheroids (ADAS) per Patient (N) by Location.

Location	AAS/N	P-value	ADAS/N	P-value	Percentage DAS (%)	P-value
Upper Medulla	22.6		5.3		23.5	
Lower Medulla	129.0	$3.3 \cdot 10^{-5}$	50.2	$3.5 \cdot 10^{-4}$	38.9	$3.8 \cdot 10^{-6}$
Spinal Cord	141.7		25.4		17.9	

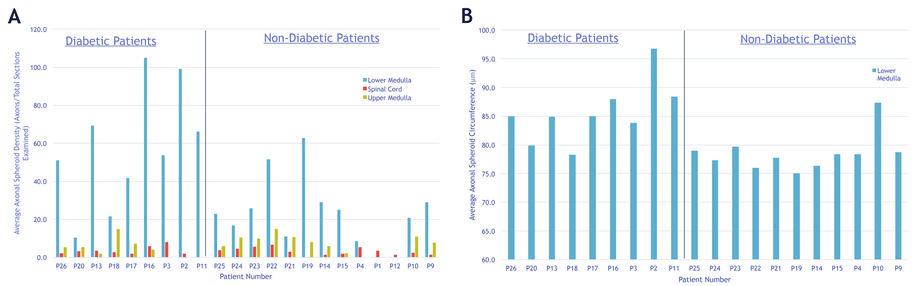

Figure 2. Average Axonal Spheroid Density and Circumference in Diabetic and Non-Diabetic Patients. (A) Average axonal spheroid density per total sections examined. (B) Average axonal spheroid circumference of each patient in the lower medulla. Upper medulla and spinal cord data not shown.

Table 4. Average Axonal Spheroid Density (AAS) in Diabetic and Non-Diabetic Patients.

Area	Patient	Density (AAS/ Section)	Standard Deviation	P-value
Upper Medulla	Diabetic	6.5	4.4	0.279
	Non-Diabetic	8.7	3.5	
Lower Medulla	Diabetic	57.6	31.6	0.013*
	Non-Diabetic	27.5	16.2	
Spinal Cord	Diabetic	3.7	2.2	0.790
	Non-Diabetic	3.5	1.8	

*Indicates p-value < 0.05

Table 5. Average Axonal Spheroid Circumference in Diabetic and Non-Diabetic Patients.

Area	Patient	Circumference (µm)	Standard Deviation (µm)	P-value
Upper Medulla	Diabetic	73.2	3.7	0.055
	Non-Diabetic	80.2	7.6	
Lower Medulla	Diabetic	85.6	5.4	0.002**
	Non-Diabetic	78.6	3.3	
Spinal Cord	Diabetic	81.7	3.3	0.288
	Non-Diabetic	79.8	4.0	

**Indicates p-value < 0.01

Discussion

Axonal spheroids are indicative of axonal injury and were identified in all patients, specifically in the region of the nucleus gracilis (Figure 1 A-D, Table 2). In contrast, other regions of the brainstem and spinal cord have a more random distribution with a low density and distribution of axonal spheroids. The regional variation was highly significant ($p=4.4 \cdot 10^{-9}$). Axonal spheroids were also graded based upon staining density and the presence or absence of eosinophilic inclusions (Figure 1 E-H). For the purposes of this study, increasing eosinophilia was hypothesized to represent more advanced stages of axonal degeneration since there was a spectrum of small pale axonal spheroids (morphologically most similar to normal axons) to large irregular hypereosinophilic spheroids. These "degenerating" axons were most closely localized to the region of the lower medulla as seen in Table 3. When comparing the density of axonal spheroids present in diabetic and non-diabetic patients, the density within the region of the lower medulla, specifically in the region of the nucleus gracilis was significantly increased (Table 4, $p=0.013$) in patients with a history of diabetes (Figure 2A). Furthermore, there is also a significant increase in circumference of axonal spheroids (Table 5, $p=0.002$) in patients with a diabetic history. The particular involvement of the nucleus gracilis in diabetic patients is most likely related to the length dependent neuropathic changes that are characteristic of diabetes in that the sensory fibers in the fasciculus gracilis that synapse upon the nucleus gracilis are the longest sensory tracts in the CNS.

There was no statistically significant correlation of density, numbers, or distribution of axonal spheroids with patient age, gender, or other documented comorbidities (data not shown). A correlation with a clinical history of hypertension could not be assessed given that 20 of the 22 patients in this study were known to be hypertensive.

Future studies will include further characterization of the axonal spheroids by electron microscopy and immunohistochemistry for advanced glycation end-products and β amyloid deposition as well as a more in-depth correlation regarding the diabetic history (severity, compliance with treatment, and length of disease).

Conclusion

- Axonal spheroids are found throughout the region of the medulla, but are concentrated in the lower medulla, specifically in the area of the nucleus gracilis.
- Patients with a clinical history of diabetes showed a significantly increased density of axonal spheroids in the nucleus gracilis compared to non-diabetic patients.
- Correlations of axonal spheroids and other co-morbidities, such as heart disease and CNS infarcts, were not statistically significant and can often be attributed to associated risk factors for these events in patients with a history of diabetes.
- Damage to peripheral axons in diabetic patients is well known but this is the first study to clearly demonstrate corresponding CNS axonal injury related to ascending sensory pathways involving the nucleus gracilis.

References

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