

## Brief Communication

# Evaluation of diabetic polyneuropathy in Type 2 diabetes mellitus by nerve conduction study and association of severity of neuropathy with serum sFasL level

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### ABSTRACT

**Introduction:** Diabetes mellitus (DM), a growing health problem globally, has reached epidemic proportions in India. Recently, Fas-mediated apoptosis has been proposed as a causative factor responsible for neuronal degeneration in diabetic polyneuropathy (DPN), but there are very few studies to show association of serum soluble Fas ligand (sFasL) level with severity of neuropathy. **Aim and Objective:** The aim of this study was to investigate whether serum sFasL, a transmembrane glycoprotein involved in apoptosis, has any association with severity of peripheral neuropathy in Type 2 DM. **Materials and Methods:** The study was conducted in Department of Physiology in collaboration with Department of Endocrinology, IPGME&R. sFasL levels in serum were assessed using ELISA method in healthy individuals ( $n = 16$ ), newly diagnosed diabetic controls ( $n = 16$ ) without any complications, and in DPN cases ( $n = 33$ ) with predominant neuropathy only. All subjects underwent both electrodiagnostic procedures and vibration perception threshold (VPT) for quantitative assessment of the severity of neuropathy. Using nerve conduction studies, amplitudes, velocities, and latencies of both sensory and motor nerves were recorded. **Results:** In DPN patients, concentration of sFasL levels ( $87.53 \pm 3.49$ ) was significantly decreased ( $P < 0.0001$ ) not only when compared with normal controls ( $225.30 \pm 2.97$ ) but also when compared with diabetic patients without any complication ( $161 \pm 3.63$ ). Moreover, the concentration of sFasL is significantly ( $P < 0.0001$ ) associated with the severity of neuropathy both by VPT and nerve conduction velocity (NCV). **Conclusion:** Fas-mediated apoptosis is involved in Type 2 DM and might be associated with the severity of polyneuropathy.

**Key words:** Diabetic polyneuropathy, nerve conduction velocity, soluble Fas ligand

## INTRODUCTION

Diabetes mellitus (DM), a growing health problem globally, has reached epidemic proportions in India. Recently, Fas-mediated apoptosis has been proposed as a causative factor responsible for neuronal degeneration in diabetic

polyneuropathy (DPN), but there are few studies to show association of serum soluble Fas ligand (sFasL) level with severity of neuropathy.

## AIMS AND OBJECTIVE

The aim of this study was to investigate whether serum sFasL, a transmembrane glycoprotein involved in apoptosis, has any association with severity of peripheral neuropathy in Type 2 DM. If positively associated with serum sFas level, it can definitely suggest a role for apoptotic dysregulation in pathophysiology of DPN. Thus, targeting and inhibiting Fas receptor offer an option for DPN therapy.

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## MATERIALS AND METHODS

The study was conducted in Department of Physiology in collaboration with Department of Endocrinology, IPGME&R. Subjects were randomly chosen and meticulous screening was done as per criteria to select cases. sFasL levels in serum were assessed using ELISA method in three groups:

- Group I: Healthy individuals ( $n = 16$ )
- Group II: Newly diagnosed diabetic controls ( $n = 16$ ) without any complications
- Group III: DPN cases ( $n = 33$ ) with predominant neuropathy only.

All subjects underwent both neuroelectrophysiological procedures using “RMS nerve conduction velocity (NCV) EMG EP MARK II” and vibration perception threshold (VPT) using “Vibrometer VPT” for quantitative assessment of the severity of neuropathy. Using standard nerve conduction techniques, amplitudes, velocities, and latencies of both sensory and motor nerves were recorded.

The results were expressed as mean  $\pm$  SD.  $P < 0.05$  was considered significant. Statistical analysis was done using the software GRAPHPAD PRISM Version 5.00 and Statistica Version 7.

## REVIEW OF LITERATURE

Recent evidences suggest that there is increased level of sFas in serum of diabetic patients with neuropathy<sup>[1]</sup> and that Fas-mediated apoptosis of neuronal cell line is likely to be responsible for neuronal degeneration in DPN as this neuropathy can be blocked by anti-Fas antibody (ZB4).<sup>[2]</sup> But till date, there is no study which shows correlation of serum sFas level with severity of DPN in Type 2 DM.

Hence, in the view increase incidence of DPN and possible role of Fas-mediated apoptosis in the etiopathogenesis of diabetic neuropathy,<sup>[1,2]</sup> we think it is quite rationale to presume that there may exist a correlation between severity of neuropathy and serum sFas level in patients of DPN in Type 2 DM.

## RESULT AND DISCUSSION

In this study, there is significant alteration in sFasL concentration among three groups [Figure 1 and Table 1a]. In Group I, the sFasL level is within normal range, i.e.,  $>0.1$  ng/ml (mean =  $225.30 \pm 2.97$ ). In Group II, sFasL concentration is significantly lower than healthy individual (Group I) (mean  $161 \pm 3.63$ )  $P < 0.0001$ , but the value is within normal range. Thus, in both Groups I and II, sFasL concentration is within normal range which has been corroborated in several other studies.<sup>[1,2]</sup> But in contrary to most of the studies who reported normal sFasL levels in DPN,<sup>[1,2]</sup> we found highly significant decrease in sFasL level in patients with DPN, the mean being  $87.53 \pm 3.49$  and intergroup statistical significance being  $<0.0001$  when compared with both healthy and diabetic controls. Two studies have reported that<sup>[3,4]</sup> decrease sFasL level in diabetic patient than healthy control and have related insulin resistance as a causal effect of decrease sFasL. This may be a common factor lowering sFasL concentration in our study. But a significant lowering of sFasL in patients with predominant neuropathy (Group III) cannot be explained only with insulin resistance. Tanaka *et al.*,<sup>[5]</sup> in 1998 in his study, reported that cleavage of membrane bound FasL (mFasL) at conserved cleavage site to generate sFasL is a mechanism to prevent apoptosis. Hence, decrease sFasL

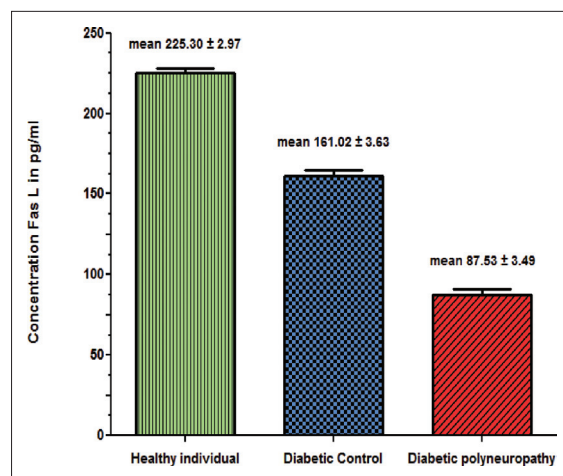


Figure 1: Comparison of soluble Fas ligand (sFasL) level among three groups

Table 1a: Comparison of serum soluble Fas ligand (sFasL) conc. with electrodiagnostic finding among three groups

Comparison groups	Concentration of FasL in pg/ml (mean $\pm$ SD)	Median		Sural		Peroneal	
		NCV in m/sec (mean $\pm$ SD)	SNAP in $\mu$ V (mean $\pm$ SD)	NCV in m/sec (mean $\pm$ SD)	SNAP in $\mu$ V (mean $\pm$ SD)	NCV in m/sec (mean $\pm$ SD)	SNAP in $\mu$ V (mean $\pm$ SD)
Group I ( $n=16$ )	225.30 $\pm$ 2.97	50.63 $\pm$ 1.02	38.78 $\pm$ 1.74	51.13 $\pm$ 0.80	29.88 $\pm$ 1.82	49.81 $\pm$ 2.13	3.13 $\pm$ 0.15
Group II ( $n=16$ )	161.00 $\pm$ 3.63	51 $\pm$ 0.71	33.56 $\pm$ 1.89	51.31 $\pm$ 1.01	23.25 $\pm$ 1.34	49.36 $\pm$ 1.36	3.01 $\pm$ 0.11
Group III ( $n=33$ )	87.53 $\pm$ 3.49	12.21 $\pm$ 2.56	6.23 $\pm$ 2.31	3.21 $\pm$ 2.86	0.88 $\pm$ 3.68	18.76 $\pm$ 2.15	1.14 $\pm$ 1.6

NCV - Nerve conduction velocity, Group I - Healthy individual, Group II - Diabetic control, Group III - Diabetic neuropathy

**Table 1b: Intergroup statistical significance of nerve conduction velocity (NCV) and Concentration of serum soluble Fas ligand (sFasL)**

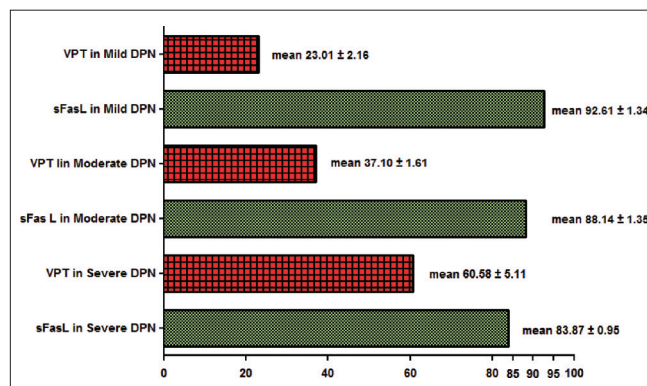
Comparison groups	Concentration of Fas L		NCV of median nerve		NCV of sural nerve		NCV of peroneal nerve	
	95% CI	P value	95% CI	P value	95% CI	P value	95% CI	P value
Group I versus Group II	61.88 to 66.68	<0.0001	-0.89 to 0.39	0.43	-0.85 to 0.47	0.56	-1.11 to 1.48	0.76
Group II versus Group III	71.32 to 75.66	<0.0001	28.25 to 49.07	<0.0001	41.58 to 54.62	<0.0001	20.66 to 41.08	<0.0001
Group I versus Group III	135.7 to 139.8	<0.0001	28 to 48.83	<0.0001	41.40 to 54.43	<0.0001	20.83 to 41.28	<0.0001

Group I - Healthy individual, Group II - Diabetic control, Group III - Diabetic neuropathy, NCV - Nerve conduction velocity

concentration in DPN may be due to increased interaction between upregulated sFas with over expressed mFas and a lesser cleavage to enhance the apoptotic activity in DPN.

Concentration of sFasL when compared with NCV finding in each group [Table 1a and b] revealed that NCV of median (sensory), sural, and peroneal (motor) was normal in Groups I and II (normal values: Median nerve: NCV =  $56.2 \pm 5.8$  m/s, Amp =  $38.5 \pm 15.6$   $\mu$ V; sural nerve: NCV =  $51.1 \pm 5.9$  m/s, Amp =  $17.2 \pm 6.7$   $\mu$ V; peroneal nerve: NCV =  $48.3 \pm 3.9$  m/s, Amp =  $5.1 \pm 2.3$  mV) without intergroup statistical significance. But there is gross decrease in both conduction velocity and amplitude in Group III subjects showing severe sensory (sural being absent in >90% cases) and moderate motor neuropathy both inducing demyelination and axonopathy. This severe neuropathy corroborates with decrease sFasL concentration as shown in Table 1a and b. This finding is in contrary to most of the studies where sFasL concentration remains normal in spite of altered electrodiagnostic finding.<sup>[1,2]</sup>

This observation, i.e., decreased sFasL has a direct correlation with severe neuropathy and is further substantiated when we compare sFasL concentration with severity of neuropathy graded according to VPT [Figure 2]. Neuropathy has been graded according to VPT as mild, moderate, and severe (mild: VPT, 20-35; moderate: VPT, 36-50; severe: VPT, >50) and it is observed that there is progressive decrease in the concentration of sFasL in these three groups of DPN. The intergroup comparison of sFasL concentration was statistically significant ( $P < 0.0001$ ). Point biserial correlation coefficient for the association between sFasL level and severity of DPN is negatively correlated [ $r_{PB} = -0.73$  ( $P < 0.001$ )]. Moreover, point biserial correlation coefficient of sFasL concentration and presence of moderate to severe VPT is also negatively correlated [ $r_{PB} = -0.78$  ( $P < 0.001$ )], showing that the severity of neuropathy is strongly associated with lowering of sFasL concentration. This is a novel finding and to our knowledge till date no study has compared sFasL concentration with the severity of neuropathy.



**Figure 2:** Alteration of soluble Fas ligand (sFasL) concentration with the severity of nerve conduction velocity (DPN) graded according to VPT [Intergroup comparison of sFasL conc. is statistically significant  $P < 0.001$ ]

Thus, we may conclude by saying that sFasL concentration decreases significantly with severity of neuropathy in Type 2 DM and may be considered a serum marker to assess the severity of DPN. But to fully establish the efficacy of this study, a larger sample size and longer follow-up are essential.

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