Original Research Article

A comparative study on estimation of salivary pH as a screening tool for identifying early neural dysfunction in type 2 diabetes

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Abstract

Background: Human saliva is a viscoelastic fluid and its secretion is basically a neurally regulated mechanism. Its flow and composition depends on the functional integrity of the nerves supplying the gland. Salivary testing works by placing an acid stress in the mouth to see if a person adapts a healthy alkaline response. **Aim:** Our study aims at identifying the early autonomic neuropathic changes in diabetes by evaluating salivary pH. The objectives were to study the change in pH of salivary secretion following an acid challenge (vitamin-c) between diabetics and controls, to correlate the magnitude of change in salivary pH with diabetic neuropathy and fasting blood glucose and to assess the influence of duration of diabetic illness on change in salivary pH. **Materials and methods:** 82 diabetic patients and 82 age-sex matched controls were tested for salivary pH before and after oral administration of vitamin-C. The change in salivary pH, fasting blood glucose level, duration of diabetes along with clinical manifestation of neuropathy were noted. Data were analyzed using SPSS. **Results:** The resting salivary pH of the diabetic and control were same. Magnitude of change in salivary ph following an acid challenge in diabetic were significantly less ($p<0.001$). Longer the duration of diabetes lesser is the the magnitude of change in salivary pH to an acid stress. **Conclusion:** A significant decrease in stimulated salivary pH following vitamin-C challenge in diabetics implies decreased salivary secretion due to autonomic blunting in diabetic patients.

Keywords: autonomic dysfunction, salivary pH, Type 2 Diabetes mellitus

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Introduction

Diabetic neuropathy is defined by the American Diabetes Association (ADA) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes".[1] Symptomatic autonomic neuropathy manifests as silent ischemia/cardiac arrest and carries a worse prognosis than any other complication of diabetes. Despite attempts to prevent its worsening through improved glycemic control and drugs, it is difficult to say whether reversal of the autonomic damage is possible by this means.[2] In research setting, the autonomic "battery of cardiovascular tests" using cardiovascular reflexes are most often done on diabetics, but it assesses autonomic neural integrity only after symptom manifestations.[2a] Subclinical autonomic nerve damage occurs more widely in diabetics than was suspected and is assuming greater importance because of high morbidity/mortality. Having considered the increasing prevalence of diabetes, efforts need to be directed at identifying a new, simple, non-invasive and inexpensive screening tool to improve preventive strategies without increasing the burden of healthcare costs.

Recent interests are towards non-invasive diagnostic testing. Salivary tests has become a highly sensitive tool in large-scale screening and epidemiological studies due to its easy collection, cost-effectiveness, ample working time to detect pathologies as it doesnot clot, making it practically feasible to diagnose and monitor diseases.[3,4] Human saliva is a complex, visco-elastic, extracellular fluid and its role starts during fetal life,[5] where its composition is important for the development of oral structures.[6-10] The concentrations of substances in saliva is almost accurate with that of the blood[11], where hormones like cortisol, sex steroids and melatonin are detectable in saliva, making it a good accessible fluid equivalent to blood.[12] Yet in humans the pH of saliva is of utmost importance as it indicates the buffering capacity of the body towards any acid foods.[13] Stimulated saliva depends on the salivary flow rate which can be directly measured from pH and is the index of salivary function.[14] Stimulation is done by placing an acid stress in the mouth and to see if a person adapts a healthy response. An alkaline response to an acid stimulus is normal and is due to an increased salivary secretion.[15,16] Salivary gland being purely under autonomic neural control, any autonomic dysfunction is reflected in its secretion.[17]

Repeated oral infections, periodontitis and bilateral parotid enlargement are frequently documented complications in diabetes mellitus (DM) and salivary dysfunction has been reported as its cause.[18.19] Since studies related to these issues are scant and their results are inadequate, this article is intended to identify a new test which is simple, non-invasive and cost-effective and also an effective indicator of autonomic nervous dysfunction. We sought to identify whether we can assess early diabetic autonomic neuropathy by testing salivary pH following an acid challenge.

The objectives of this study were to estimate the stimulated and unstimulated salivary pH following an acid challenge (vitamin-c) between diabetic patients and controls, to correlate the magnitude of change in salivary pH with diabetic neuropathy, to assess the influence of the duration of diabetic illness on change in salivary pH, to correlate the magnitude of change in salivary pH with fasting blood glucose levels of diabetic patients.

Materials and Methods

After getting institutional ethics clearance and written informed consent, this study was done in the Institute of Diabetology, Madras Medical College, Chennai.

Selection of subjects: A simple random sampling of 164 subjects of 30-60 years of age of both sexes, in which 82 clinically diagnosed type 2 diabetic patients were selected from in-patients of diabetology ward and 82 age and sex matched clinically healthy subjects were selected from master health-check up, Madras Medical college, Chennai. **Exclusion criteria:** Subjects with previous history of any connective tissue disorder, cerebro-vascular disorders, oro-dental disorders, other neurological disorders like seizures, past history of any surgical illness and other metabolic disorders like thyroid, vit-B12 deficiency, folic acid deficiency and those who were smokers, alcoholics, tobacco chewers were excluded. **Materials used:** 1. pH strips (sigma): standardized with a sensitivity range of 4.5 – 9.0 are very accurate, user friendly and practitioner recommended. It can be used to test urine or saliva.

Reliability of the pH strips: A single sigma pH strip was dipped in 10 ml of distilled water and it showed a pH of 7. It was tested with 3 samples of 10ml distilled water where it produced the same result of pH 7. An adequate amount of the same distilled water was prepared and used for our study.

2. Colour chart showing the pH range is matched with the pH strip.
3. Acid challenge was given orally as chewable vitamin - C tablets (500mg of ascorbic acid).
4. Distilled water was used to rinse mouth before starting the test.

Procedure: Procedure was clearly explained and the socio-demographic details, duration of illness, the fasting blood glucose levels were collected from the subjects. All subjects were examined clinically for any dental or mucosal abnormalities to ensure that there is a relatively good oral hygiene and a patent salivary duct opening.

Collection of samples: Samples were collected from subjects during morning in empty stomach. All subjects were instructed not to consume any food, tablets or drinks including tea or coffee (except for water). After brushing teeth or using of mouth wash solutions, one hour time gap was ensured since the composition of the tooth paste or the solution may influence the study. All subjects were asked to rinse their mouth with distilled water half an hour before the test. Subjects were asked to keep open their mouth for 2-3minutes. Under strict aseptic precautions, the pH strip was placed on the undersurface of the tongue near the frenulum and the pH of the unstimulated human saliva was collected. pH of saliva was noted by matching the pH strip with the colour chart provided. The subjects were then asked to chew the vitamin-C tablet and not to spit-out/swallow the tablet until it is completely dissolved. After 5 minutes of complete dissolution of the tablet, subjects spat out the saliva and pH(stimulated saliva) was again noted.

Assessment of neuropathy: Diabetic neuropathy was assessed using Diabetic Neuropathy Symptom (DNS) score, where a score ≥ 1 was considered significant (Annexure I) and Diabetic Neuropathy Examination (DNE) score (Annexure II) where a score > 3 was considered significant for presence of neuropathy(10). Areas affected by ulceration or thick callus formation were omitted. With patients eyes closed, 10g Semmes-Weinstein monofilament was applied to the plantar surface of great toe and base of first, third, and fifth metatarsals of both foot. The patient was asked to say(yes/no) when he feels the pressure applied and in which foot it was applied. Inability to perceive the sensation at any one site was considered abnormal. Vibration sensation was tested with tuning fork(128Hz) applied at the bony prominence of both legs. Patients were examined for ankle reflex.

Statistical analysis: A statistical analysis was performed using SPSS and p< 0.05 was considered significant. Paired 't' test was used to compare the salivary pH before and after vitamin-C administration in both the diabetic and control subjects. Unpaired 't' test was used to compare the magnitude of change in salivary pH between diabetics and controls. Pearson correlation was used to find any correlation between the duration of diabetic illness and the magnitude of change in salivary pH in the study group.

Results

The mean age of diabetic patients and control subjects were (52.7 ± 18) and (45.66 ± 11) respectively. The magnitude of change in salivary pH was calculated from the difference of pH between the resting unstimulated salivary pH (pH before vitamin-c administration) and the stimulated salivary pH (pH after vitamin-C administration), i.e: Magnitude of change in salivary pH = resting unstimulated salivary pH - stimulated salivary pH.

I) Comparison of the resting unstimulated salivary pH and stimulated salivary pH following an acid challenge between the diabetic group and the control group:

The data are shown in Table 1. No significant differences in the resting salivary pH were found between both diabetics (5.1 ± 1.4) and controls (5.8±1.5). The increase in pH of the stimulated salivary secretion after vitamin-C administration is significantly less in diabetic patients (5.8 ± 0.8) than that of the controls (8.3 ± 1.7) as in Table.1.

II) Comparison of the magnitude of change in salivary pH between diabetic patients and controls:

The magnitude of change in salivary pH (resting unstimulated salivary pH - the stimulated salivary pH) is highly significant with lesser difference (<3) in diabetics than the controls (>3) implying that the ability of the saliva to neutralize the acid challenge is decreased in diabetic patients. Almost 96% of diabetic individuals showed a difference of pH <3 when compared to the control study group (difference of pH > 3) as shown in Figure 1.

III) Comparing the presence of neuropathy and the magnitude of change in salivary pH in the diabetic study group: Among the diabetic patients, 53 had clinical manifestations of diabetic neuropathy, and their magnitude of change in salivary pH found to be below 2.5 as shown in Figure 2. The more severe the neuropathy, the very less is the magnitude of change in salivary pH. i.e the stimulated salivary pH remained acidic. In the remaining 29 diabetic subjects without any symptoms of neuropathy, 17 subjects showed an acidic stimulated salivary pH where their magnitude of change in pH was ≤2. Whereas the 12 out of 29 diabetic subjects without neuropathy had a normal alkaline response after salivary stimulation.

IV) Correlation between the duration of diabetic illness and the magnitude of change in salivary pH in the diabetic study group: A significantly strong negative correlation was found between the duration of diabetes (mean duration of 6.5±1.4years) and magnitude of change in salivary pH as shown in Figure 3. As the duration of diabetes is longer, lesser is the magnitude of change in salivary pH i.e.the stimulated salivary pH is less. In our study, no significant correlation was found between the fasting blood glucose levels and the magnitude of change in salivary pH.

Discussion

In our study we made an honest attempt of testing the salivary pH following an acid challenge with vitamin-C chewable tablet in type-2 diabetic patients and healthy control subjects. Without stimulus salivary glands inherently secrete saliva.[20] Unstimulated saliva is the salivary secretion at rest. Stimulated saliva done with strong stimuli like citric acid, where the parotid secretes more and its flow rate equals to that of submandibular gland.[21] During chewing, its flow rate becomes twice as high as that of submandibular gland. Salivary secretion is about 1–2 L/day. The relative contributions are roughly 60% from submandibular glands, 30% from parotid glands, 10% from sublingual and other minor glands.[22] Low salivary flow occurs at night; it can be increased at rest by low-grade mechanical stimuli(movements of the tongue and lips,mucosal dryness).[23] Though the circadian rhythm of salivary flow in individuals is still a matter of debate, fact that salivary flow is less at early morning than at noon is considered.[24] Hence we preferred collecting samples at early morning hours.

We observed the resting unstimulated salivary pH as slightly acidic (pH=5.0-6.5) in both the diabetic patients and the healthy control subjects(table.1). It might be due to the early morning sample collection in an empty stomach or might be a reflection of oral flora. But the estimation of stimulated salivary pH after vitamin-C administration showed a significant difference between the controls and diabetics, which played a crucial evidence in our study. After administering vitamin-C to control subjects, we observed an increase in salivary pH which was becoming overtly alkaline(pH=8-9.5). Reason could be due to an increased salivary secretion caused by the acid stimulus to neutralize it. Whereas in diabetic patients, after administering vitamin-C, though an increase in pH(from 5-5.5 to 5.5-6) was noted it remained still as acidic pH. This substantiates an inadequate salivary secretion and a strong evidence for the reduced buffering capacity by the salivary gland. Though a detailed evaluation on salivary flow was not done, salivary pH is considered as the direct reflectant of salivary secretion. Also it has been reported in literature that the low saliva pH of T1DM patients is strong evidence of reduced buffer salivary capacity and increased caries risk.[16] They explained the low unstimulated salivary flow contributed to the low pH values observed. In agreement to the present study results, a study reported significantly lower salivary pH(unstimulated and stimulated) in patients with type 1 and type 2 DM as compared to the normal subjects. This effect could be secondary to decreased salivary flow rates.[25] In contrary, some studies did not find any significant differences in salivary flow rates and some reported that only stimulated salivary pH was reduced in diabetic patients.[26-28]

Among the patients, in those who were clinically diagnosed of diabetic neuropathy, we observed their stimulated salivary pH to be acidic(Figure 2). Some diabetic patients had no neuropathy manifestations and showed a normal alkaline response after vitamin-C administration. Surprisingly, few diabetics in our study showed an acidic pH after acid stimulus but without any other manifestations of neuropathy. This shows the possibility of decreased salivary secretion due to autonomic blunting and gives us a clue that they might develop neuropathy in near future. Their salivary secretion might be inadequate to combat an acid stress and this can be an early sign of subclinical autonomic dysfunction. However, we also observed that longer the duration of diabetic illness(mean duration of 6.5±1.4years), limited is the secretory ability of the salivary gland to neutralize the acid stress(Fig.III). Pathophysiological changes could be due to ischemic damage or demyelinating changes in the nerves supplying the glands.[29-30] Studies have proposed that the duration of illness have impact on the demyelination, thickeneing of vessel walls and desensitization of receptors in type2 diabetes mellitus.[31-33]

How prolonged exposure to high blood glucose can cause nerve damage in diabetic patients is still an area of research. We found a more decreased stimulated salivary pH in uncontrolled diabetic patients. However, we did not find any direct correlation between fasting blood glucose levels and the change in salivary pH. Reuterving et al. did not find any significant difference in pH, buffer capacity, proteins, electrolytes, lysozymes, peroxidases of saliva.[34] They concluded that the degree of metabolic control doesn’t have a great influence in salivary composition, except in the salivary concentration of glucose. Nevertheless, a decrease in stimulated salivary pH observed in patients with type2 diabetes mellitus can be supported with more evaluation of salivary flow rate and its buffer capacity. A detailed evaluation of the salivary flow rates was not carried out. Dodds et al. studied the effects of metabolic control in salivary flow of type 2 diabetics finding a markedly decreased salivary flow.[35]

Implications:

Salivary pH testing using pH-strips can be a simple, cost-effective bedside tests that can say whether autonomic damage is present or not. In our study, the salivary pH appeared as a possible correlate of neuropathy in diabetes. Qualitatively assessing the Salivary pH with an acid-challenge can be used for screening purpose. Limitations of the study: More subjects from various populations needed in order to develop our observations into a definitive methodology to monitor disease onset and progression. And neurodiagnostic tests must be done to confirm diabetic neuropathy and to substantiate our results. Future perspective: We intend to focus on ways to assess the salivary flow rate and scintigraphy in diabetics. The long-term aim of management should be the identifying autonomic damage in its early stages using salivary pH testing at outpatient department.

Conclusion

There is a definite autonomic blunting as evidenced by a decreased response to acid stress in type 2 diabetic individuals. Decreased magnitude of change in salivary pH to an acid stress is observed with the presence of neuropathy and with the longer duration of the illness in diabetic patients. Though a detailed evaluation is mandatory. Salivary pH testing can be a simple, cost effective, non-invasive screening tool in identifying early neural dysfunction.

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Conflicts of interest: Nil

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Table 1 : Resting salivary pH, Stimulated salivary pH and the magnitude of Change in Salivary pH between the diabetics and control group

Study	Variables	Mean	Standard Deviation	Sig.(2-tailed)
Type2DM (n=82)	pH before vitamin-C administration	5.1	1.4	0.000*
	pH after vitamin-C administration	5.8	0.8	
	Magnitude of Change in Salivary pH	2	0.7	
Controls (n=82)	pH before vitamin-C administration	5.8	1.5	0.000*
	pH after vitamin-C administration	8.3	1.7	
	Magnitude of Change in Salivary pH	4.1	0.5	
Control Vs Diabetic	pH before vitamin-C administration	-0.3	0.7	0.005**
	pH after vitamin-C administration	1.3	0.94	0.001**
	Magnitude of change in pH	2.1	1.14	0.000**

Type2DM – Type 2 Diabetes Mellitus;p<0.05 = significant;*paired t test, ***independent t test.

Figure 1: Comparison of the magnitude of change in salivary pH between diabetic patients and controls.

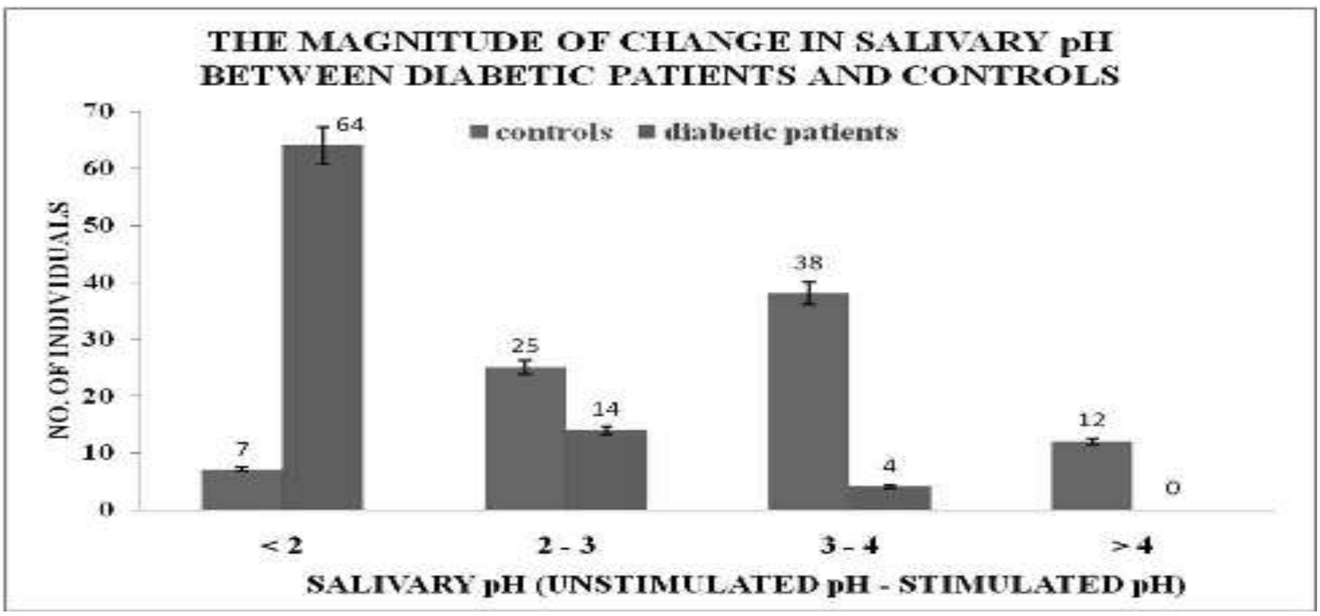


Figure 2: Comparing the presence of neuropathy and the magnitude of change in salivary pH in the diabetic group.

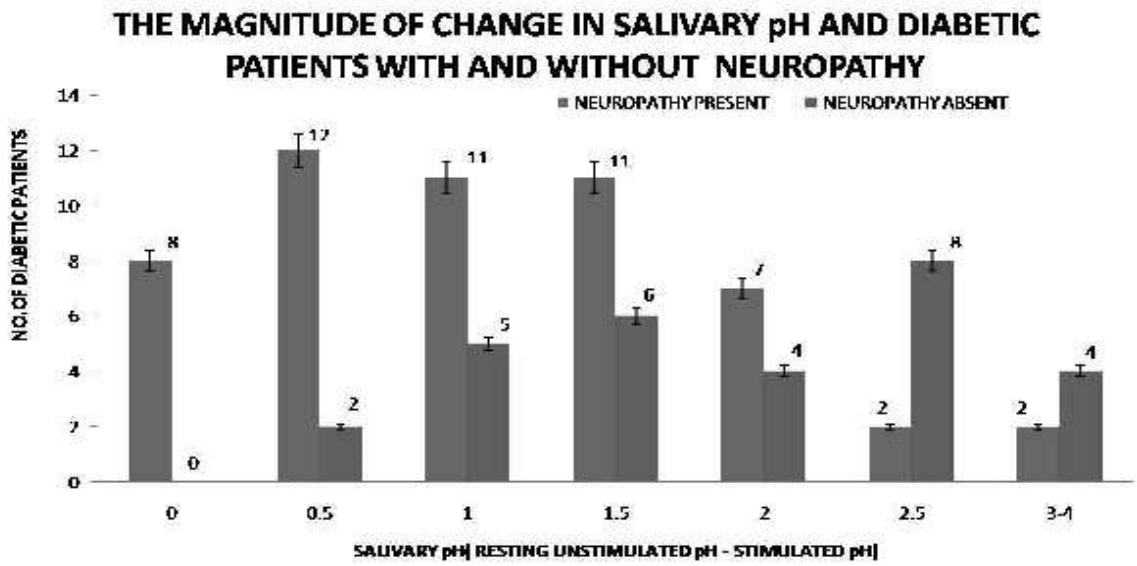
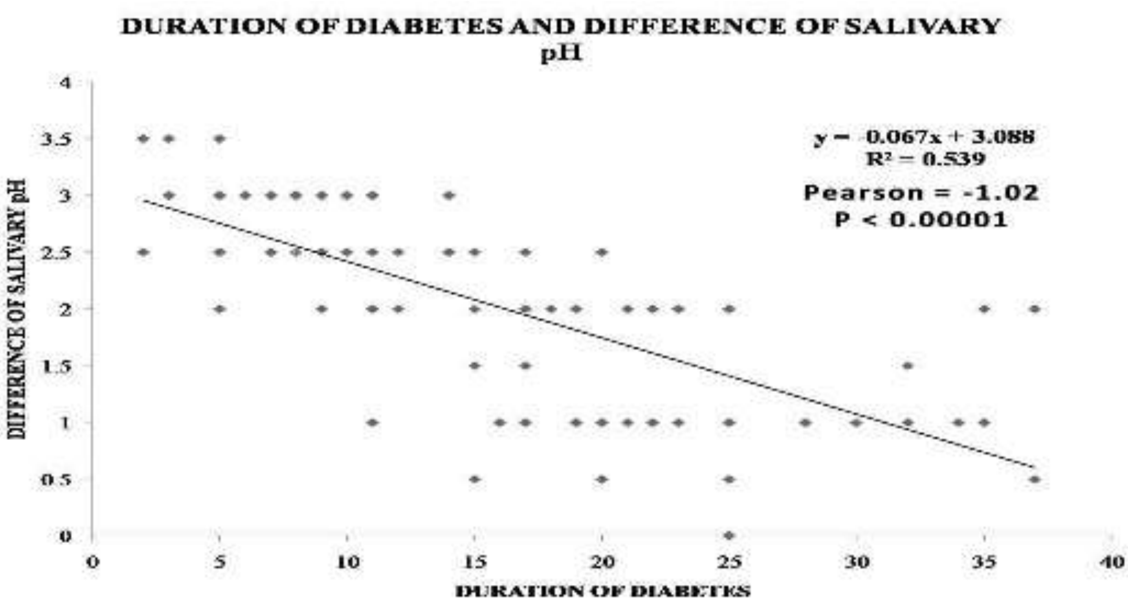


Figure 3: Correlation between the duration of diabetic illness and the magnitude of change in salivary pH in the diabetic group.



ANNEXURES:

Annexure I

DNS Questionnaire

1. Are you suffering of unsteadiness in walking? *Need for visual control, increase in the dark, walk like a drunken man, lack of contact with floor.*

2. Do you have a burning, aching pain or tenderness at your legs or feet? *Occurring at rest or at night, not related to exercise*, *exclude intermittenent claudication.*

3. Do you have prickling sensations at your legs and feet? *Occurring at rest or at night, distal>proximal, stocking glove distribution.*

4. Do you have places of numbness on your legs or feet? *Distal>proximal, stocking glove distribution.*

The questions were answered either 'Yes' (positive: 1 point) if a symptom has occurred during the last 2 weeks or 'No' (negative: no point) if it did not. Maximum score is 4 and minimum 0.

Annexure II

DNE scoring

Muscle strength:

1. Quadriceps femoris: extension of the knee 2. Tibialis anterior: dorsiflexion of the foot.

Reflex: 3. Triceps surae

Sensation: index finger: 4. Sensitivity to pinpricks

Sensation: big toe: 5.Sensitivity to pinpricks, 6.Sensitivity to touch, 7.Vibration perception, 8.Sensitivity to joint position

Only the right leg and foot are tested.

Scoring from 0 to 2:

0 = Normal

1 = Mild/moderate deficit: Muscle strength: Medical Research Council scale 3–4, Reflex: decreased but present, Sensation: decreased but present

2 = severely disturbed/absent Muscle strength: Medical Research Council scale 0–2, Reflex: absent, Sensation: absent

Maximum score: 16 points