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. Our study aims at identifying the early autonomic neuropathic changes in diabetes by evaluating salivary gland function. Salivary testing works by placing an acid stress in the mouth to see if a person adapts a healthy response. A highly alkaline reserve to an acid stimulus is a normal response. AIM: To study the change in pH of salivary secretion in diabetic individuals following an oral acid challenge (vitamin-c) and to compare it with controls. To assess whether the duration of diabetes influences the change in pH. To correlate the diabetic neuropathy with the change in salivary pH.

Materials and method: 81 diabetic individuals and 54 age matched controls were tested for salivary pH before and after oral challenge of vitamin-C using a standard pH strip. Their fasting blood glucose level, the type of diabetes and the duration of diabetes along with their clinical manifestation were recorded. The data were evaluated and analyzed and their significance determined using the appropriate T test.

Results: The resting salivary pH of the diabetic and the control were almost same. The magnitude of change in salivary pH following an oral vitamin-C challenge were significantly less (p<0.001) compared to that of the controls. Longer the duration of diabetes lesser is the the magnitude of pH change to acid stress. No significant correlation between the blood glucose level and the magnitude of change in salivary pH.

Conclusion: There is a significant decrease in salivary pH change following an oral vitamin-C challenge in diabetics indicating an autonomic blunting. Sensitivity of the test is 96% and specificity of the test is 75%. Thus estimation of salivary pH is a simple, effective, non-invasive method in identifying early neuronal impairment in diabetes mellitus and offers a cost-effective method for screening of large population.

Keyword: Saliva, pH strips, Vitamin-C, Diabetic patients

ESTIMATION OF SALIVARY PH BEFORE AND AFTER AN ACID STIMULUS IN DIABETIC PATIENTS

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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. Our study aims at identifying the early autonomic neuropathic changes in diabetes by evaluating salivary gland function. Salivary testing works by placing an acid stress in the mouth to see if a person adapts a healthy response. A highly alkaline reserve to an acid stimulus is a normal response. AIM: To study the change in pH of salivary secretion in diabetic individuals following an oral acid challenge (vitamin-c) and to compare it with controls. To assess whether the duration of diabetes influences the change in pH. To correlate the diabetic neuropathy with the change in salivary pH.

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1. INTRODUCTION: Human saliva is a complex, visco-elastic, extracellular fluid secreted mainly by the three major salivary glands parotid, submandibular and sublingual. The study of saliva has been overwhelmingly difficult by its enormous naturally occurring variability and the regularity of the composition of the fluid. The distinct functions of saliva is because of its biophysical properties constituted by organic and inorganic substances, glycoprotein, lipids and digestive enzymes suspended in an aqueous medium."
The most prominent buffering agents in saliva are bicarbonate and phosphate ions and these agents protect the oral health. Thus, the salivary pH is a fair indicator of the health of extra-cellular fluids and the alkaline mineral reserves. In a balanced individual the optimum pH of saliva is 6.8-7.2. After eating the saliva pH should rise to 7.8 or higher. The pH levels of saliva is of critical importance as it indicates the buffering capacity of the body towards any acid foods entering into the system. Salivary pH reflects functional integrity of salivary secretion (Reuvering et al., 1987) and is basically under the control of autonomic nervous system.

Salivary gland function. 5,6 Salivary testing works by placing an autonomic neuropathic changes in diabetes by evaluating sensation, gangrene, etc. Autonomic neuropathy preceeds distal sensory motor neuropathy presenting as ulcer, loss of glandular neuro-regulation produced by demyelination. 4. Diabetes mellitus is known to cause salivary gland dysfunction. 3. The type 2 DM (non-insulin dependent) or adult diabetes usually affects people aged above 30. This metabolic syndrome is characterised by qualitative and quantitative deficits of insulin that is reflected in the glucose metabolism. Most of the non-insulin dependant diabetic patients present with complications clinically. 6 The most common complication is distal sensory motor neuropathy presenting as ulcer, loss of sensation, gangrene, etc. Autonomic neuropathy preceeds peripheral neuropathy. Our study aims at identifying the early autonomic neuropathic changes in diabetes by evaluating salivary gland function. 5,6 Salivary testing works by placing an acid stress in the mouth to see if a person adapts a healthy response. A highly alkaline reserve to an acid stimulus is a normal response.

**2. AIM:**
A. To study the change in pH of salivary secretion in diabetic individuals following an oral acid challenge (vitamin-c) and to compare it with controls. 
B. To assess whether the duration of diabetes influences the change in pH. 
C. To correlate the diabetic neuropathy with the change in salivary pH.

3. MATERIALS AND METHODS: Standard pH strips 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. Colour chart showing the pH range is matched with the pH strip. Acid challenge was given orally as chewable vitamin - C tablets (500mg of ascorbic acid). Distilled water was used to rinse mouth before starting the test. The study was conducted in the Institute Of Physiology And Experimental Medicine, Madras Medical College, Chennai. The study protocol was approved by our Institutional Ethical Committee, Madras Medical College, Chennai. The subjects participating in this study were informed about the study and written consent was obtained from them before including them in the study. This study is a cross sectional study and subjects were randomly selected using random number table. The cases participated in this study were in-patients of diabetology ward and consisted of 81 type 2 diabetic patients with mean age (52.7 ± 12) among which 50% are male and 50% are females. The age matched control group consisted of 54 clinically healthy people (26 females/28 Males) with mean age (50.66 ± 11). The control groups did not have any health problem except for occasional medications with vitamins and analgesics. After explaining and getting an informed consent, the subjects who were non-smokers, non-alcoholics and non-dental abnormalities to ensure that there is a relatively good oral hygiene and a patent salivary duct opening. Among the cases the type 2 diabetic patients

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The resting salivary pH was almost same in both diabetics and controls. The pH of the stimulated salivary secretion after an acid challenge is less in diabetics (7.02 ± 0.81) than that of the controls (8.3 ± 0.35). The magnitude of change in salivary pH following vitamin – C administration is significantly decreased in diabetics compared to that of controls as shown in fig. 1. In fig. 1 almost 96% (39±39) of diabetic individuals showed a difference of pH <3 when compared to controls (difference of pH >3).

From the review of literature not much studies have used salivary pH as a tool to diagnose neuropathy in diabetes. Some supportive evidences for the role of autonomic nervous system in salivary secretion like researchers from Portugal investigated the effects of diabetes mellitus (types I and II) on human salivary gland function compared to healthy age-matched controls. The results have shown that both type I and type II diabetic patients secrete significantly less resting and stimulated saliva compared to healthy age-matched controls. These results indicate that diabetes mellitus can lead to marked dysfunction of the secretory capacity of the salivary glands. In these patients a modified fluid, organic and inorganic salivary secretion may be responsible for the increased susceptibility to oral infections as concluded by Mata and co-authors. In our study the resting salivary pH and the magnitude of pH change following an acid challenge in diabetic individuals showed a similar decrease although the resting salivary pH of the controls was also less.

Diabetes mainly affects the autonomic, peripheral and central nervous system, among which autonomic neuropathy precedes peripheral neuropathy. However the symptoms of distal sensory motor neuropathy are clinically manifested earlier. The cause for neuropathy could be various factors like metabolic, prolonged duration of diabetes, low levels of insulin, autoimmune, inflammatory and inherited. In our study we did find there is a significant (p < 0.0001) inverse relationship between the duration of diabetes and the magnitude of pH change. The pathophysiological changes could be due to excess glucose entering polyol pathway and this leads to sorbitol and fructose accumulation in the nerves causing impaired axonal transport and nerve conduction thus damaging the nerve fibres. Having irregular or elevated blood glucose levels for many years can damage the blood vessels that bring oxygen to the nerves. The small capillaries in the nerves are damaged with thickening of basement membrane, formation of infiltrates leading to thickening of the vessel wall. This leads to ischemia of the nerves and can also cause demyelination. Researchers are studying how prolonged exposure to high blood glucose causes nerve damage. There was no relationship between blood glucose and salivary pH in our study. On the other hand there are several confounding factors associated with studies attempting to correlate other parameters like fasting blood glucose levels, duration of diabetes and the salivary pH.

Low levels of insulin have been proposed to have impact on demyelination, thickening of vessel walls and desensitization of receptors. In our study, the decreased change of salivary pH following vitamin-C administration in diabetics shows that there is poor buffering capacity of saliva. This diminished buffering capacity of saliva can be associated with flow-reducing salivary glandular pathologies due to structural or neurological derangement in diabetes. Furthermore, we consider that the salivary secretion in non-insulin dependant diabetic patients was not sufficient enough to neutralise the given acid stress exemplifying a definite autonomic blunting. This consideration is based on the fact that the salivary secretion is purely under neural control and the diabetes affects the nerves primarily. Supporting evidence to our study has been taken from an article stating that “Parasympathetic denervation causes progressive atrophy of striated ducts and oedematous degeneration of some cells in salivary glands. Sympathetic denervation causes a loss of apical secretory material between 2–4 days”. This implies that autonomic blunting will affect the salivary glandular function. Another important fallout from our study was that 30 out of 81 diabetic patients showed a definite clinical evidence of neuropathy.
the remaining although did not show overt manifestations of neuropathy did show a decreased response to acid challenge proving that this simple test could be used as a diagnostic tool in identifying subclinical neuropathy.

7. CONCLUSION:
There is definite autonomic blunting as evidenced by a decreased response to acid stress in diabetic individuals. Longer the duration of diabetes, lesser is the magnitude of pH change to acid stress. Thus salivary pH test is a simple, non-invasive and effective method in identifying early diabetic neuropathy because it is very easy to collect and offers a cost-effective method for screening of large population. Apart from that it can also offer an excellent alternative for serum for diagnostic purposes thus ensuring a quick, accurate diagnosis of neuropathy which can limit nerve damage and preserve the patient’s function and sensation.

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9. REFERENCES: