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Evaluation of Salivary Nerve Growth Factor Levels in Type II Diabetics with Peripheral Neuropathy

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ABSTRACT

Purpose: Application of growth factors such as nerve growth factor (NGF) is sometimes used to regenerate and reconstruct neurons in diabetic peripheral neuropathy (DPN), but requires close monitoring of serum NGF. Here, we tested saliva of patients with diabetes mellitus with neuropathy for NGF as the basis for a less-invasive monitoring method.
Materials and Methods: This case-control study was carried out on 82 patients (23 controls, 29 diabetics without neuropathy and 30 diabetics with neuropathy) treated at the Diabetic Research Center in the city of Hamadan, Iran, each of whom gave unstimulated saliva samples. Salivary NGF was measured by EIA. The data were analyzed by chi-square, t-test, Tukey and non-parametric equivalent tests.
Results: Salivary NGF in the control group was higher than in diabetics with neuropathy ($P = 0.048$) and diabetics without neuropathy ($P = 0.015$). However, salivary NGF in the two diabetic groups did not significantly vary ($P = 0.872$).
Conclusion: Variation in salivary NGF may act as a helpful marker for DPN.
KEY WORDS: nerve growth factor, saliva, diabetes, diabetic neuropathy

INTRODUCTION

Diabetes mellitus is the most common endocrinopathy. It afflicts about 9% of the world's population.[1] In Iran, the rate of diabetes is close to 5.5%. Diabetes includes a series of metabolic disorders whose common feature is high blood sugar. Type II diabetes is the most common and includes 90–95% of all cases.[2] One common disorder is diabetic peripheral neuropathy (DPN) which affects 50% of diabetes patients.[3] This disorder generally affects small sensory fibers which are mainly distributed in the arms and legs.[4] Histologically, this disorder shows up by degeneration, demyelination and axonal atrophy, along with regeneration and unsuccessful demyelination of neurons.[5] DPN is the second most common factor in amputation of legs after accidents, and is a factor in about half of all non-traumatic leg amputations. Also, DPN is reported to be the most important reason for hospitalization in these patients.[6]

Methods of regenerating and remineralizing axons damaged in DPN are being widely investigated. One such effort uses growth factors, such as Nerve Growth Factor (NGF),[7] a well-studied neurotrophin that mediates development and maintenance of the peripheral nervous system.[8] However, such factors must be monitored, as they can precipitate metabolic disorders and affect reconstruction vascular growth factors and apoptosis.

Various studies have shown that maintenance and growth of sympathetic neurons and sensory neurons in the vertebrate posterior spinal ganglion depend on the presence of NGF, and these neurons are generally affected in DPN. In fact, interaction and reaction of neurons with growth factors released from target tissues are associated with improved performance and maintenance.[9] Some studies suggest that the rate of NGF declines in peripheral neurons of mice treated with streptozotocin (STZ), which attacks pancreatic beta cells and can be used to induce diabetes in animal models.[10,11] Kim et al showed levels of serum NGF in diabetics with neuropathy was higher than in subjects without neuropathy. However, NGF is used to treat DPN.[3]

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Saliva is increasingly used as a diagnostic index, as it is easier to collect and prepare than serum. Diabetes can affect the flow and components of saliva; these changes can cause symptoms and even disorders.[2] We therefore investigated the relationship between levels of salivary and neuropathic NGF as a basis for future treatment of DPN. Accordingly, the aim of this study is to analyze the level of NGF in saliva of patients with DPN.

MATERIALS AND METHODS

Patients

In this case-control study, subjects were selected from patients referred to the Hamadan Diabetes Research Center, and the control group from Hamadan Dentistry University. Type II diabetes was diagnosed using ADA13. The study included 30 patients suffering from neuropathic Type II diabetes, 29 non-neuropathic diabetic patients and 23 people as the control group were chosen for the study. Diabetic subjects were 45–70 years of age; all had histories of ≥10 years as diabetics. Subjects with neuropathy had it for ≥ 1 year. Neuropathy was diagnosed and classified using the Michigan Neuropathy Screening Score,[14] which included a series of quantitative neurologic examinations and a group of 5 of nerve conduction studies.[15] All participants gave written informed consent. Demographic data was gathered using a questionnaire. The three groups were matched based on age, gender and other intervening factors. Patients were excluded if they had other neurologic disorders which might have presentations similar to neuropathy. Criteria to enter the study included cranial neuropathies, non-symmetrical proximal neuropathies, levels of aspartate aminotransferase, alkaline phosphatase, or bilirubin twice the normal level, and a history of immunosuppressive treatment, any defect in immune system, smoking, coagulative disorders, and patients with idiopathic neuropathy as well as Type II diabetic patients who used insulin or other blood sugar-controlling medicines.

Methods

Each participant provided 5 cc of non-stimulated saliva by spitting for 10 minutes. [3] Saliva was collected from 9–11 am with participants in resting positions. Participants abstained from eating and drinking one hour prior to the collection and washed their mouths with water just before collecting the saliva. Samples then were put in Falcon tubes and kept at a temperature of ⁻20°C until testing. After collecting all samples, they were defrosted and centrifuged for 10 minutes at 4°C at 800 g so epithelial cells and cell debris were separated and saliva density was reduced.

Levels of saliva NGF were measured in duplicate through a commercial enzyme-linked immunoassay (EIA) and with the use of Human NGFß Wuhan Boster Biological Technology Co., Ltd kit, code: Ek0469 Lot No: 188858218 made in China.

Statistics

The diabetic and control groups were compared using X^2 and *t*-tests. Data was analyzed using SPSS software, version 16, through chi-square, t-test and complementary Tukey test, and nonparametric equivalent tests. $P < 0.05$ was considered significant.

RESULTS

We studied 82 people, ranging in age from 47 to 70 years. Table 1 shows gender, age, HbA1c, BMI, FBS, blood pressure and history of diabetes. The number of people who used insulin is shown in Table 3. Survey results indicated that patients with DPN were older than diabetics without neuropathy (DM/N⁻), and they had higher BMI, blood pressure, FBS, HbA1c and longer histories of diabetes, and more of them used insulin. The controls, DPN and DM/N⁻ groups did not significantly differ by gender (X^2 test= 0.600, $P = 0.692$ between DM/N⁻ group and controls; X^2 test= 0.000, $P = 0.100$ between the DPN group and controls).

The results showed no significant difference among the three groups in regard to BMI ($P = 0.722$ for the DM/N⁻ group and controls; $P = 0.414$ for the DPN group and controls). The *t*-test showed no significant difference between patients with diabetes and controls ($P = 0.528$ between the DM/N⁻ group and controls; $P = 0.497$ between the DPN group and controls).

Average salivary NGF in the control group was significantly higher than in the other two groups ($P = 0.048$ and $P = 0.015$, respectively) (Tables 2, 3) but did not vary significantly between the DPN and DM/N⁻ groups ($P = 0.827$).

DISCUSSION

Neuropathy is a major complication of diabetes. Although our understanding of the pathogenesis and treatment of DPN have improved greatly in recent years, further research is needed to find more effective methods to prevent its onset, complications and progression.[16]Early in the development of DPN, axonal regeneration, remyelination, and synaptogenesis appear to fail, accompanied by increased apoptosis, possibly mediated by growth factors. Neurotrophic factors are required for the maintenance of the neurons. NGF, which is the best-studied neurotropic factor, has a critical role in protecting neurons against apoptosis and degeneration. The hypothesis that reduced levels or activity of NGF in diabetes plays a significant role in DPN pathogenesis is well-supported.[9] Steinbacher et al. have shown NGF levels in rats with STZ-induced diabetes are greatly reduced in the superior cervical ganglion.[17] Hellweg et al. also concluded that retrograde transport of NGF in the sciatic nerve to decline in rats with STZ-induced diabetes.[18]Anand's study found NGF is reduced in epidermal keratinocytes in human diabetic skin and this decrease was accompanied to dysfunction of cutaneous sensory fibers. These data imply that changes in availability of NGF may be responsible in part for early small-fiber neuropathy.[11] Pittenger et al. showed that alterations in endogenous blood concentrations of NGF may be associated with hyperglycemia and/or diabetes, and these changes may be effective to the development of neuropathy.[9] Similar to this study, Ordonez reported that NGF concentrations were reduced in the serum in rats with STZ-induced diabetes compared with matched controls.[19]

In 2011,Tiaka showed that NGF levels are reduced in foot skin of diabetic patients in the early neuropathic stages.[20] These articles agree with the present study, which indicates a significant decrease in salivary concentrations of NGF in diabetic patients compared to those of controls, and among the diabetic groups, NGF levels in patients with DPN was higher, but not significantly, than those of patients with DM/N⁻. Low levels of NGF could be due to either decreased production or transport of NGF in diabetes or both, possibly as a result of glucose-induced oxidative stress.[9]

Although NGF levels were analyzed in nerve ganglia, sciatic nerve, skin, serum and salivary glands, changes in saliva concentration of NGF have been measured in this study.

Nam et al. (2007) studied NGF concentrations in saliva from healthy people and evaluated the effects of age and sex differences on salivary NGF. Their findings showed saliva to have measurable concentrations of NGF. Levels of NGF were affected by the source for the stimulated parotid and submandibular saliva, age for stimulated submandibular saliva, and gender for resting whole saliva and stimulated parotid saliva.[21]In a study by Jang et al. associations between pain intensity and concentration of NGF in plasma and saliva of chronic migraine patients was analyzed. They concluded that increased NGF production affects maintenance of pain in chronic migraine; moreover saliva neuropeptide levels correlated very well with their plasma levels, which suggests the possibility of utilizing saliva as a less invasive diagnostic tool for measuring pain markers of chronic migraine.[22]

In their articles, Nam and Jang evaluated the effect of age and gender in measuring NGF in saliva and revealed that saliva NGF concentration is significantly higher in females; furthermore, collection times, and collection methods of saliva in research might contribute to the different concentrations.[21,24] For these reasons, gender, time and method of sample collection as confounding factors were all synchronized in this study.

In contrast to experiments delineated above, Kim`s study (2009) to determine NGF levels and activity of NGF receptors in DPN patients, reported diabetics with neuropathy had markedly increased serum and tear NGF levels compared with diabetics without neuropathy and nondiabetic controls. However, in Kim`s study, serum NGF levels gradually and significantly decreased according to severity of neuropathy.[3]

In our study, NGF levels tended to be higher in neuropathic patients than in non-neuropathic subjects, but not significantly so. The relation between salivary NGF level and severity of neuropathy was not analyzed in this study; similarly, Kim explicitly said his study could not establish any clear relationship between changes in NGF level and development of diabetic neuropathy. Possibly, increased NGF production is a compensatory reaction to decreased neuron activity and decreased serum NGF.[3] Compensatory NGF elevation has been reported in Alzheimer disease, after an initial deficit of NGF at the onset of the pathological process.[27] Neuropathy caused by alcohol has been also reported. [25,26]

Considering this study and similar surveys[21], changes in salivary NGF level may be helpful as a less-invasive diagnostic tool for monitoring NGF to represent neuropathic complications.

Conclusion

Saliva concentration of NGF has a negative association with the development of diabetes. Although the salivary NGF level was higher, but not significantly so, in diabetic patients with neuropathy than those without neuropathy.

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Table 1: Demographic characteristics of subject groups.

	Healthy controls	Diabetic with neuropathy	Diabetic without neuropathy
Sex(female/male %)	10/14 (43.47)	14/15 (48.27)	16/23 (53.3)
Age(years)	60.8±11.9	62.7±10.0	57.1±11.9
FBS(mg/dL)	–	197.25	190.15
HbA1c(%)	–	9.2	8.5
Disease duration(years)	–	15.1	11.2
BMI(Kg/m2)	25.6	26.8	26.1
Insulin (%)	–	69.89	4.47
Blood Pressure (mmHg)	–	137±13	130±1

Table 2: Minimum, maximum, mean and standard deviation of salivary nerve growth factor in study groups

Groups	Number	Minimum	Maximum	Mean	SD*	95% CI
Controls	23	250.00	710.00	429.13	126.09	374.60–483.65
DM/N¯	29	220.00	500.00	351.71	75.07	323.16–380.28
DPN	30	220.00	500.00	364.33	90.54	330.52–398.14
Total	82	220.00	710.00	378.05	101.29	355.79–400.30

*Standard Deviation
DM/N¯: diabetes mellitus without neuropathy; DPN: diabetic peripheral neuropathy.

Table 3: Comparisons of salivary nerve growth factor level in different groups

Groups	Mean differences	Standard error	95% CI for mean	*P*
C vs DM/N¯	77.40	27.12	142.18–12.62	0.015
C vs DPN	64.79	26.92	129.10–0.149	0.048
DPN vs DM/N¯	¯12.60	25.29	47.81–¯73.02	0.872

C: Control; DM/N¯: diabetes mellitus without neuropathy; DPN: diabetic peripheral neuropathy.