

Neuropathy in Prediabetics: Is Oxidative Stress to Contribute?

Amrinder Singh¹, Ajay Chauhan^{2*}, Parul Goyal³, Priyamvada Ramesh¹

Abstract

Objective: To assess the association of oxidative stress and serum vitamin D levels in sensory neuropathy in prediabetes.

Method: Serum and urine levels of 8-OHdG (a marker of oxidative stress) and serum levels of vitamin D were compared in prediabetic patient having sensory neuropathy to those who did not have sensory neuropathy as determined by VPTs measured by Digital Biothesiometer and MNSI (Michigan Neuropathy Screening Instrument)

Result: A total of 60 prediabetic cases between 35 years to 60 years were included in this study. Among all the prediabetic subjects, 43.3 % subjects had neuropathy according to VPTs measured by Biothesiometer. T-test analysis suggested that serum levels of 8-OHdG were significantly higher in subjects with neuropathy than subjects without neuropathy (1006.58 ± 511.8 vs 688.6 ± 607.3 , p value = 0.035). Urinary levels of 8-OHdG were also significantly higher in subjects with neuropathy than subjects without neuropathy (699.35 ± 419.5 vs 474.57 ± 402.5 , p-value = 0.04). No such significant difference however was present in serum levels of vitamin D between neuropathic and non-neuropathic prediabetics (20.13 ± 18.44 vs 16.96 ± 11.72 , p value = 0.419). VPTs were found to have statistically significant positive correlation with serum 8-OHdG {Pearson Correlation Coefficient= 0.317(R), 0.307(L); p-value=0.014(R),0.017(L)} and urine 8-OHdG levels{Pearson Correlation Coefficient= 0.288(R), 0.255(L); p-value=0.026(R), 0.049(L)}. According to MNSI physical assessment score (> or = 2), 38.3 % subjects (23 subjects) had neuropathy. MNSI score is positively correlated with serum 8-OHdG (Pearson Correlation Coefficient = 0.308; p-value = 0.017). Correlation with urine 8-OHdG was not statistically significant (Pearson Correlation Coefficient= 0.687; p value = 0.06). Correlations of MNSI scores {Pearson Correlation Coefficient=0.14, p-value=0.287} and VPTs{Pearson Correlation Coefficient=0.058(R), 0.189(L); p-value=0.660(R), 0.148(L)} with serum vitamin D levels were not statistically significant.

Conclusion: Oxidative stress, as confirmed by the biomarker, 8-OHdG, has a important role in the development of this sensory neuropathy.

Introduction

Type 2 DM is characterized by a long asymptomatic phase (ranging from 4 to 7 years) between the genuine onset of hyperglycemia and clinical diagnosis which may explain the relatively high prevalence of microvascular complications in newly diagnosed patients of type 2 DM.¹

Emerging body of evidence suggests that the pathophysiological process of these microvascular and even macrovascular complications, to some extent, starts as early as in prediabetic stage.² Impaired glucose tolerance

(IGT)/ prediabetes is a marker of insulin resistance and is predictive of microvascular and macrovascular complications, irrespective of progression to diabetes.³

In a study done in India in 2014, neuropathy was detected in 32.8% subjects with IGT (prediabetes). Thus, the peripheral neuropathy may be much more common in prediabetics than previously thought.⁴ In another

study done in 2015, the prevalence of peripheral neuropathy was 50%, 49%, and 29% for new-onset diabetes, prediabetes, and normal glycemia, respectively.⁵ Going by all the above information and data, it's very likely that the process of microvascular complications like neuropathy starts well before the onset of overt diabetes. Conventional wisdom of diabetic peripheral neuropathy being caused by advanced glycation end products is giving way to newer emerging knowledge which lays great emphasis on the role of oxidative stress in the pathogenesis of diabetic neuropathy. This has even led to clinical trials of antioxidants such as α -lipoic acid, (a powerful antioxidant that scavenges hydroxyl, superoxide and peroxy radicals and regenerates glutathione) in the treatment of diabetic neuropathy.⁶

8-OHdG (8-Hydroxy-2'-deoxyguanosine) is one of the major by-products of DNA oxidation.⁷ Serum and urinary 8-OHdG are being used as a novel biochemical marker of oxidative damage in various newer studies in many diseases including atherosclerosis, diabetes, prediabetics, colorectal cancers etc. Other factors like vitamin D deficiency have also been implicated in pathogenesis of diabetic peripheral neuropathy in various studies. The aim of the present study is to assess serum and urine levels of 8-OHdG, and serum vitamin D levels as predictors of occurrence of sensory neuropathy in prediabetics.

Materials and methods

Place of study

The study was conducted in the Departments of Medicine and Biochemistry at PGIMER and Dr. Ram Manohar Lohia hospital, New Delhi

¹Postgraduate Resident of Medicine, ²Professor of Medicine, ³Professor of Biochemistry, PGIMER, Dr. RML Hospital, New Delhi;

*Corresponding Author

Received: 27.08.2018; Accepted: 05.04.2019

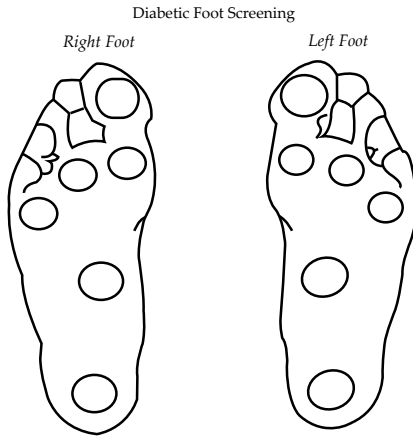


Fig. 1: Areas to measure VPTs in PLANTAR method

during the study period between November 2016 and March 2018.

Study Design

A cross sectional observational study was performed.

Sample size

A total of 60 prediabetic (as defined by American Diabetes Association) subjects were evaluated.

Selection of study population

The target population consisted of patients of prediabetes either admitted as In-patient (IPD) or visiting the Outpatient Department (OPD) between November 2016 and March 2018. 60 consecutive patients fulfilling all inclusion and exclusion criteria were included in the study.

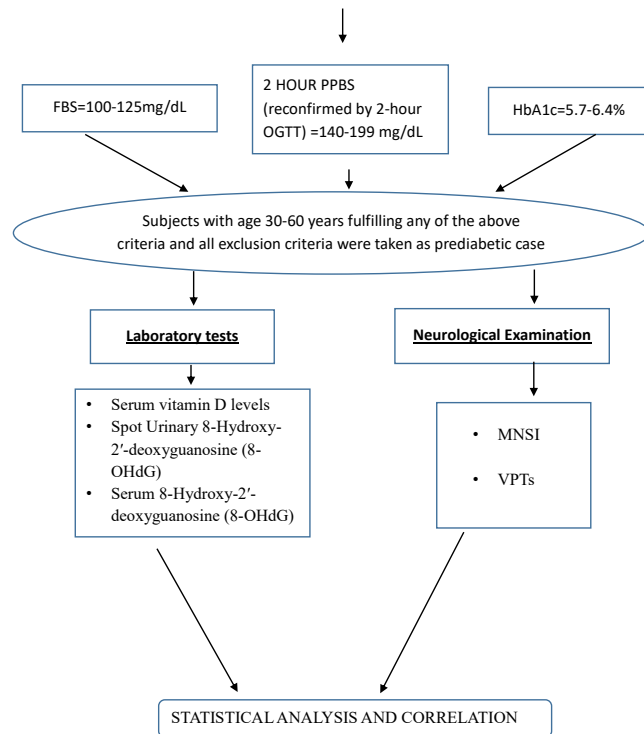
Inclusion criteria considered were:

- Age of 30-60 years
- Fasting plasma glucose between 100 to 125 mg/dL or
- 2-hour postprandial plasma glucose between 140 to 199 mg/dL [were included in the study only after reconfirming with standard 2-hour OGTT (after 75 gm of glucose solution ingestion)] or
- HbA1c =5.7-6.4%

Exclusion criteria were cases of:

- Cerebrovascular accidents
- Hypothyroidism
- Chronic alcoholics
- Patients on anti-tubercular treatment and other drugs known to cause neuropathy
- Smokers
- Patients on chemotherapy and/or Radiotherapy

ALGORITHM



Algorithm 1: Statistical analysis and correlation

- SLE
- Leprosy
- Vasculitis
- Malignancy
- Neurological disorders like Guillain-Barré syndrome, Multiple Sclerosis
- HIV +ve patients
- Chronic glucocorticoid therapy
- Vitamin B12 deficiency.

Levels of Serum 8-OHdG, urine 8-OHdG serum vitamin D, and vibration perception thresholds (VPT)⁸ were measured along with calculation of MNSI physical assessment score in all the subjects.

The spot urine samples and fasting serum samples for 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and Vitamin D were collected and centrifuged at 3000rpm for 10 minutes. For estimation of urine and serum 8-OHdG, the supernatant was immediately aliquoted and stored at -20°C till batch analysed by ELISA on EVOLIS Twin Plus by Biorad. Serum Vitamin D levels were measured by Enhanced Chemiluminescence on ECiQ, by Orthoclinical Diagnostics.

VPTs were measured by Digital Biothesiometer (Vibrotest) using PLANTAR method (Figure 1) and

average value was calculated in both feet. Biothesiometer is a useful diagnostic tool for quantitatively grading the neuropathy, manufactured by Diabetik Foot Care India pvt Ltd. Subjects with average VPT of >15 V on either side were considered to have neuropathy.

MNSI (Michigan Neuropathy Screening Instrument)⁹ local extremity examination was also performed in this study and score >2 was considered as peripheral sensory neuropathy.

Data were analysed for statistical correlation of serum Vitamin D levels, serum 8-OHdG levels, urine 8-OHdG with both the neuropathy parameters. A brief summary of methodology is given in algorithm 1.

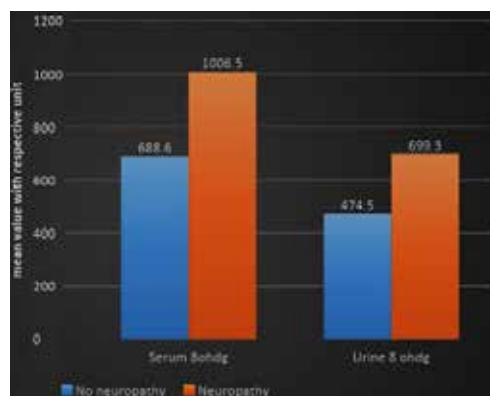
Data analysis

The Data obtained were entered in Microsoft Excel Worksheet. Statistical analysis was done using statistical software package SPSS v22.0. Data is represented as mean± SD. Mean value of continuous variable was compared using t-test and nominal variables were compared using chi square test. Pearson's correlation coefficient was calculated to assess the correlation between two continuous variables.

P-value <0.05 was taken as statistically significant.

Results

A total of 60 prediabetic cases were included in this study. Among study population, the age distribution ranged from 35 years to 60 years with the mean age of 48.68 years. Male and female formed 65% and 35% of study population respectively. The maximum serum Vitamin D levels were 95.3 ng/ml and the minimum were 8.0 ng/ml with the mean value being 18.3 ± 14.9 ng/ml. The maximum Serum 8-OHdG levels were 2882.4 pg/ml and the minimum were 105 pg/ml with the mean value being 826.4 ± 583 pg/ml. The maximum Urine 8-OHdG levels were 1974.9 pg/ml and the minimum were 82 pg/ml with the mean value being 571.975 ± 421.7 pg/ml (Table 1). Among all the prediabetic subjects, 43.3% subjects had neuropathy according to VPTs measured by Biothesiometer. T-test analysis suggested that serum levels of 8-OHdG were significantly higher in subjects with neuropathy than subjects without neuropathy (1006.58 ± 511.8 vs 688.6 ± 607.3 , p value = 0.035). Urinary levels of 8-OHdG were also significantly higher in subjects with neuropathy than subjects without neuropathy (699.35 ± 419.5 vs 474.57 ± 402.5 , p-value = 0.04) (Graph 1). No such significant difference however was present in serum levels of vitamin D between neuropathic and non-neuropathic prediabetics (20.13 ± 18.44 vs 16.96 ± 11.72 , p value = 0.419 (Table 2). VPTs were found to have statistically significant positive correlation (Tables 3 and 4) with serum 8-OHdG {Pearson Correlation Coefficient= 0.317(R), 0.307(L); p-value=0.014(R),0.017(L)}



Graph 1: Bar diagram showing comparison between 8-OHdG levels in subjects with or without neuropathy

and urine 8-OHdG levels {Pearson Correlation Coefficient= 0.288(R), 0.255(L); p-value=0.026(R), 0.049(L)} (Graphs 2, 3, 4, 5). According to MNSI physical assessment score (> or = 2), 38.3% subjects (23 subjects) had neuropathy. MNSI score is positively correlated with serum 8-OHdG (Pearson Correlation Coefficient = 0.308; p-value = 0.017) (Graph 6). Correlation with urine 8-OHdG was not statistically significant (Pearson Correlation Coefficient=0.687; p value = 0.06) (Graph 7). Correlations of MNSI scores {Pearson Correlation Coefficient=0.14, p-value=0.287} and VPTs {Pearson Correlation Coefficient= 0.058(R), 0.189(L); p-value=0.660(R), 0.148(L)} with serum vitamin D levels were not statistically significant.

Discussion

Prediabetes is considered to be a precursor phase of diabetes mellitus in which blood sugar levels are higher than normal but not high enough to meet the criteria of diabetes mellitus. Thus, this intermediate stage is often referred to as the grey area or grey zone between normoglycemia and overt diabetes. Prediabetic neuropathy is a new emerging entity. There is paucity of literature pertaining to peripheral neuropathy in prediabetes, esp. from India. Therefore, this study was designed to understand the role of various above-mentioned biomarkers and neuropathy in prediabetes.

Subjects were assessed for occurrence of neuropathy by MNSI physical assessment score and by measuring VPTs using Biothesiometer. Statistical correlations of neuropathy in prediabetic subjects with serum and urine 8-OHdG levels, and serum vitamin D levels were studied.

VPT results have been found to have good correlation with NCV findings. Klima RR et al reported comparison of VPT values, based upon whether or not evoked sensory and motor responses were obtained in NCV, indicated that mean VPTs were consistently higher among subjects in whom these evoked responses were not elicited.¹⁰ Catherine L. Martin et al reported that VPT was a sensitive predictor of DSPN, with the highest sensitivity noted for confirmed clinical neuropathy (87%). The sensitivity of VPT to predict definite clinical neuropathy and abnormal nerve conduction was 80 and 75%, respectively.¹¹ P. Jayaprakash et al quoted that Vibration perception threshold (VPT) is considered as a gold standard for diagnosis of diabetic peripheral neuropathy.¹² Hence, VPTs can reliably detect sensory neuropathy and serve as a useful, less time-consuming and less painful alternative to nerve conduction studies.

8-Hydroxydeoxyguanosine (8-OHdG) is a novel biochemical marker of oxidative stress which is formed by oxidative DNA damage following

Table 3: Pearson's correlation of VPT R (Right side) with 8-OHdG levels

Parameter	Pearson correlation	Value
Serum 8-OHdG	Pearson correlation	0.317
	Sig. (2-tailed)	0.014
	N	60
Urine 8-OHdG	Pearson Correlation	0.288
	Sig. (2-tailed)	0.026
	N	60

Table 4: Pearson's correlation of VPT L (Left side) with 8-OHdG levels

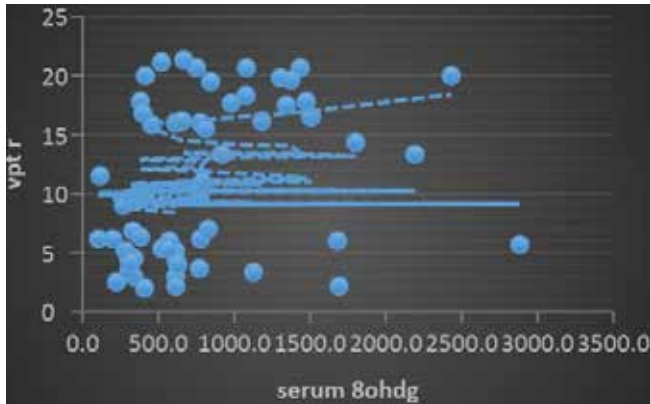
Parameter	Pearson correlation	Value
Serum 8-OHdG	Pearson correlation	0.307
	Sig. (2-tailed)	0.017
	N	60
Urine 8-OHdG	Pearson correlation	0.255
	Sig. (2-tailed)	0.049
	N	60

Table 1: Baseline characteristics

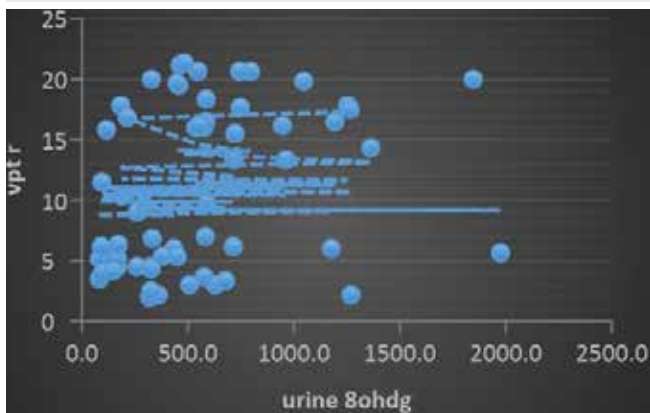
	N	Minimum	Maximum	Mean±SD
Age (years)	60	35	60	48.68±7.782
Serum Vit. D levels (ng/ml)	60	8	95.3	18.3±14.9
Serum 8-OHdG (pg/ml)	60	105.0	2882.4	826.405±583.0078
Urine 8-OHdG (pg/ml)	60	82.0	1974.9	571.975±421.7249

Table 2: Comparison of biochemical parameters in subjects with or without neuropathy

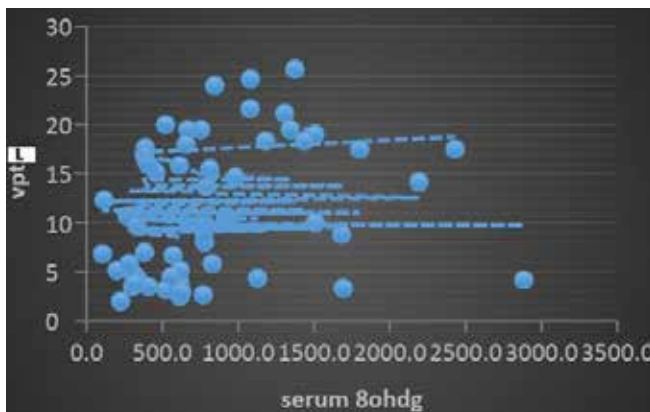
	Neuropathy	N	Mean ± SD	p-value
Vitamin-D	No	34	16.96±11.72	0.419
	Yes	26	20.13±18.44	
Serum 8-OHdG	No	34	688.6±607.3	0.035
	Yes	26	1006.58±511.8	
Urine 8-OHdG	No	34	474.57±402.5	0.04
	Yes	26	699.35±419.5	



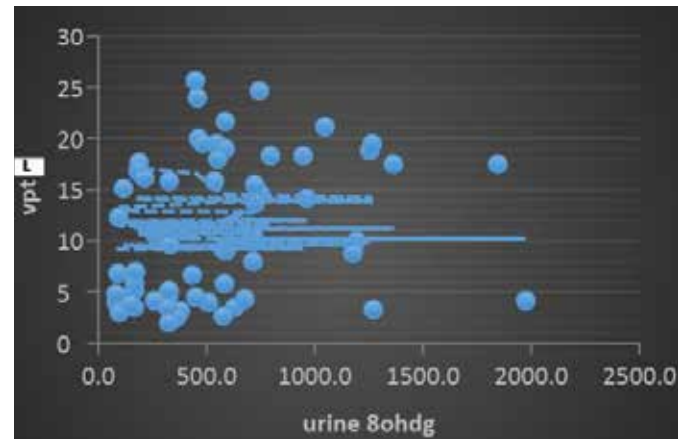
Graph 2: Correlation between VPT R and serum 8-OHdG



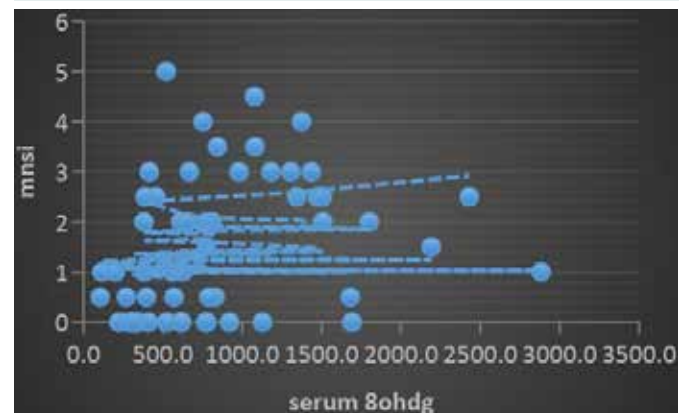
Graph 3: Correlation between VPT R and urine 8-OHdG



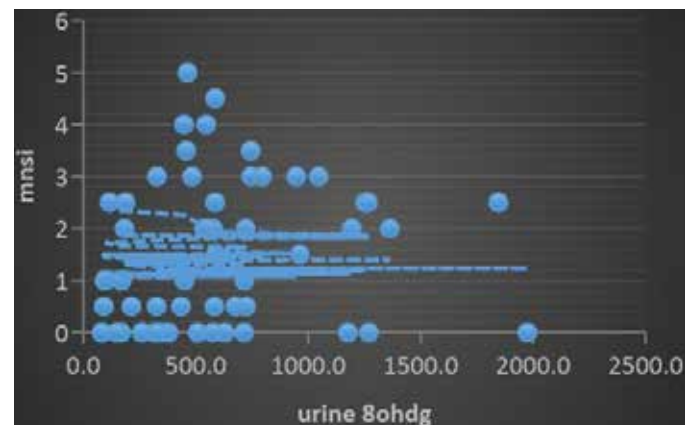
Graph 4: Correlation between VPT L and serum 8-OHdG



Graph 5: Correlation between VPT L and urine 8-OHdG



Graph 6: Correlation between MNSI score and serum 8-OHdG



Graph 7: Correlation between MNSI score and urine 8-OHdG

specific enzymatic cleavage after ROS induced 8-hydroxylation of the guanine base in mitochondria and nuclear DNA. Leinonen J et al found presence of elevated levels of 8-OHdG in urine in NIDDM patients, especially in patients with poor blood glucose control.¹³ This reflects the ongoing DNA damage in uncontrolled hyperglycaemia. In another article, by Valavanidis A et al, 8-OHdG was described as a critical marker of oxidative stress as well as carcinogenesis.¹⁴

There is growing evidence to

suggest that the role of vitamin D is not confined to calcium and phosphate homeostasis. In recent years, there has been interest among researchers to identify other target organs affected by vitamin D. Hypovitaminosis D is highly prevalent in patients with Type 2 diabetes and could be a prelude to the development of diabetic neuropathy as deficiency of vitamin D contributes to the development of neurotrophic deficits. There was no statistically

significant correlation between serum vitamin D levels and sensory neuropathy in prediabetic subjects in our study. However, in contrast to this finding, Shehab D et al, in a study done in 2012, found that Vitamin D deficiency is an independent risk factor for diabetic peripheral neuropathy.¹⁵ On reviewing previous literature, we found that relationship between this variable and sensory neuropathy has not been studied previously in prediabetic cases.

As per our knowledge, this is the first study to investigate the role of serum vitamin D levels in pathogenesis of sensory neuropathy in prediabetes.

Serum and urine 8-OHdG levels were found to be significantly higher in prediabetics with neuropathy (p-value < 0.05). Their levels also significantly correlated with sensory neuropathy in prediabetic subjects (p-value < 0.05). This is well supported in a study by Chan Soo Shin et al who found that Diabetic patients, especially those with advanced microvascular complications, had significantly higher serum 8-OHG levels; suggesting that oxidative damage plays a vital role in the development of microvascular complications of diabetes like neuropathy, nephropathy and retinopathy.¹⁶ We already know that microvascular complications like neuropathy are caused by direct endothelial injury, production of advanced glycation end products and oxidative stress. With the help of the novel biomarker 8-OHdG, our study further consolidated the role of oxidative stress in peripheral neuropathy in prediabetes. This biomarker has not been previously studied for its role in sensory neuropathy in prediabetes and our study is first to underscore the significance of this marker and consequently the role of oxidative damage in neuropathy in prediabetes. Though the occurrence of neuropathy in prediabetes has been studied, but as per our knowledge, there are no published studies, in Indian medical literature dwelling upon the roles of the

probable causative factors of sensory neuropathy in prediabetes.

Conclusion

To sum it all up, sensory neuropathy, like in diabetes, also occurs in prediabetic stage. Oxidative stress, as confirmed by the biomarker, 8-OHdG, has a role to play in the development of this sensory neuropathy. Further larger studies are required to elaborate upon this further.

Glossary of abbreviations

8-OHdG: 8-hydroxy-2'-deoxyguanosine; ADA: American Diabetes Association; DM: Diabetes Mellitus; DNA: Deoxyribonucleic acid; ELISA: Enzyme-linked Immunosorbent Assay; HIV: Human Immunodeficiency Virus; IGT: Impaired Glucose Tolerance; MNSI: Michigan Neuropathy Screening Instrument; SLE: Systemic Lupus Erythematosus; VPT: Vibration Perception Threshold; OGTT: Oral Glucose tolerance test

References

1. Yadav S, Ramesh V, Bhatia E, Gill H. A prospective study of prevalence and association of peripheral neuropathy in Indian patients with newly diagnosed type 2 diabetes mellitus. *Journal of Postgraduate Medicine* 2014; 60:270.
2. Elizabeth LM, Tien Y, Robyn J et al. Is peripheral neuropathy associated with retinopathy and albuminuria in individuals with impaired glucose metabolism? *Diabetes Care* 2006; 29:1114-1116.
3. Singleton J, Smith A, Russell J, Feldman E. Microvascular Complications of Impaired Glucose Tolerance. *Diabetes* 2003; 52:2867-2873.
4. Kannan M, Sarva S, Kandadai R, Paturi V, Jabeen S, Borgohain R. Prevalence of neuropathy in patients with impaired glucose tolerance using various electrophysiological tests. *Neural India* 2014; 62:656.
5. Lee C, Perkins B, Kayaniyl S, Harris S, Retnakaran R, Gerstein H et al. Peripheral Neuropathy and Nerve Dysfunction in Individuals at High Risk for Type 2 Diabetes: The PROMISE Cohort. *Diabetes Care* 2015; 38:793-800.
6. Diabetic Neuropathy—A Review [Internet]. Medscape. [cited 2018 May 3]. Available from: <https://www.medscape.com/viewarticle/558574>
7. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. *Journal of Environmental Science and Health, Part C* 2009; 27:120-139.
8. Maffei L, Premrou V, Roldan P, Copetti M, Pellegrini F, Rossi M, et al. Vibration Perception Threshold in the Screening of Sensorimotor Distal Symmetric Polyneuropathy. *Journal of Diabetes Science and Technology* 2014; 8:621-622.
9. Herman W, Pop-Busui R, Braffett B, Martin C, Cleary P, Albers J et al. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabetic Medicine* 2012; 29:937-944.
10. Klima RR e. Nerve conduction studies and vibration perception thresholds in diabetic and uremic neuropathy. - PubMed - NCBI [Internet]. <http://www.ncbi.nlm.nih.gov/pubmed/1848989?report=abstract>
11. Martin C, Waberski B, Pop-Busui R, Cleary P, Catton S, Albers J, et al. Vibration Perception Threshold as a Measure of Distal Symmetrical Peripheral Neuropathy in Type 1 Diabetes: Results from the DCCT/EDIC study. *Diabetes Care* 2010; 33:2635-2641.
12. Jayaprakash P, Bhansali A, Bhansali S, et al. Validation of bedside methods in evaluation of diabetic peripheral neuropathy. *The Indian Journal of Medical Research* 2011; 133:645-649
13. Leinonen J, Lehtimäki T, Toyokuni S, Okada K, Tanaka T, Hiai H, et al. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Letters* 1997; 417:150-152.
14. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. *Journal of Environmental Science and Health, Part C* 2009; 27:120-139.
15. Shehab D, Al-Jarallah K, Mojiminiyi O, Al Mohamedy H, Abdella N. Does Vitamin D deficiency play a role in peripheral neuropathy in Type 2 diabetes?. *Diabetic Medicine* 2011; 29:43-49.
16. Shin C, Moon B, Park K, Kim S, Park S, Chung M, et al. Serum 8-Hydroxy-Guanine Levels Are Increased in Diabetic Patients. *Diabetes Care* 2001; 24:733-737.