PREVALENCE OF CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS

Samuel Dinesh Alfred¹, Sukanya Nadinarajan², Sindhiya Jayachandran³, Pravin Selvam Selvaraj⁴, Santhi Chinnaiyan⁵, Kishore Raja Subramanian⁶, Vignesh Nariyamattu Chandrakumar⁷, Shivamalarvizhi⁸

¹Associate Professor, Department of Internal Medicine, Stanley Medical College.

²Assistant Professor, Department of Internal Medicine, Stanley Medical College.

³Postgraduate, Department of Internal Medicine, Stanley Medical College.

⁴Postgraduate, Department of Internal Medicine, Stanley Medical College.

⁵Postgraduate, Department of Internal Medicine, Stanley Medical College.

⁶Intern, Department of Internal Medicine, Stanley Medical College.

⁷Intern, Department of Internal Medicine, Stanley Medical College.

⁸Postgraduate, Department of Internal Medicine, Stanley Medical College.

ABSTRACT

BACKGROUND

Diabetic neuropathy, a set of clinical syndrome sometime silent and undetected, may be single or combined with signs which are nonspecific, insidious and slow and often diagnosed by exclusion. Neurologic complications occur equally in all types of diabetes – type I, type II and all other types of diabetes.

MATERIALS AND METHODS

This study aimed at assessing the prevalence of Cardiac Dysautonomia in type 2 diabetic individuals using conventional testing methods and by using Aniscope.

RESULTS

'At diagnosis' 68.8% were CAN positive by aniscope and 81.2% by conventional method. In individuals with a duration of 1 - 5 years, 82% and 88% tested positive for autonomic dysfunction by aniscope and conventional method respectively.

CONCLUSION

Diabetics for more than 5 years were tested 100% positive for autonomic dysfunction by either of the methods.

KEYWORDS

Diabetes, Aniscope, Cardiac Autonomic Neuropathy.

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BACKGROUND

Diabetes: A Challenge of 21st Century

Change in the lifestyle of people along with globalisation in the past century has increased the incidence of diabetes. Findings of a report by Ramachandran et al has proved⁽¹⁾ urbanisation of India causing a high prevalence of diabetes. It has increased from 13.9 in 2002 to 18.2 in 2006 in urban areas. The rural area also showed an increase in prevalence from 6.4 in 2002 to 9.2 in 2006. According to International Diabetic Federation Diabetes Atlas,⁽²⁾ India is next only to china hosting the high number of people with diabetes. The number of people with diabetes is expected to increase from 65.1 million in 2013 to 109 million in 2035 in India. Indians develop diabetic complications at an early age. This results in increase in mortality and morbidity among Indians. Despite a high prevalence of diabetes in South-East Asian countries, only 5% of the global health care cost goes towards diabetes care.

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Corresponding Author:

Dr. Samuel Dinesh Alfred,

No. 404, Shruthi Block, Chitra Avenue,

Choolaimedu High Road,

Chennai-94.

E-mail: meyappansindhi@gmail.com

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Prevention of complications associated with diabetes is achieved by primary prevention by modifying risk factors, such as insulin resistance and obesity.⁽³⁾⁽⁴⁾

Type 2 diabetes is a disorder characterised by insulin resistance, relative decrease in insulin secretion and hyperglycaemia. Environmental and genetic factors play a role in the development of diabetes.

Diabetic neuropathy, a set of clinical syndrome sometime silent and undetected, may be single or combined with signs which are non-specific, insidious and slow and often diagnosed by exclusion. Neurologic complications occur equally in all types of diabetes – type I, type II and all other types of diabetes.

Diabetic neuropathy is the most common cause of hospitalisation than other known cause of complications. So early diagnosis of cardiac autonomic neuropathy by using simple non-invasive investigation – heart rate variability using ECG helps in the identification of individuals at risk. This study aimed at assessing the prevalence of Cardiac Dysautonomia in type 2 diabetic individuals using conventional testing methods and by using mathematical instrument - Aniscope.⁽⁵⁾

MATERIALS AND METHODS

Patients with Type 2 diabetes in both OP and IP basis from Feb. 2013 to Dec. 2014 were included in the study. Patients were subjected to symptom analysis, clinical examination and laboratory investigations.

Eligible cohorts were subjected to standard autonomic testing and testing with Aniscope.

Standard Autonomic Testing includes,
Assessing Heart Rate Variability with

1. Deep breathing.
2. Valsalva.
3. Supine-to-standing position.

Assessing Blood Pressure Variability with

1. Supine-to-standing.

Procedure

Resting ECG was taken for all the patients. Individuals in the study group were subjected to ECG recordings. Preferred lead is lead II.

Subjects were made to lie supine comfortably. Then they were asked to take deep breathe evenly at the rate of 6 breaths per minute, i.e. 5 seconds for inspiration and 5 seconds for expiration. A continuous ECG was recorded for one minute. The maximum and minimum R-R interval during the respiratory cycle was calculated and converted to beats per minute. The difference between the two and heart rate variation of less than 10 beats per minute was taken as abnormal. Then the patient was allowed to lie quietly for another 5 minutes. The patient was made to exhale forcibly into the mouth piece of manometer sustaining a pressure of 40 mmHg for about 15 seconds and the ECG was recorded continuously. The patient was made to stop the manoeuvre and the ECG was further recorded post manoeuvre. The ratio of shortest R-R interval during the manoeuvre and the longest R-R post valsalva was calculated. A ratio less than 1.10 was considered abnormal. Again the patient was made to lie supine quietly. After about 5 minutes with continuous monitoring, the patient was made to stand. The R-R interval at 15th beat and 30th beat was calculated. The 30:15 ratio of less than 1.00 was considered abnormal.

For the assessment of the sympathetic function, patient was made to lie down and his BP recorded. Then he was made to stand up and again BP measurement was made 2 minutes after standing. A fall of systolic Blood Pressure more than 30 mmHg was considered abnormal. Based on the above standard testing, patients with 2 or more abnormal tests were classified as definite, one of the three heart rate variability test abnormal were classified as early. When individuals with parasympathetic dysfunction along with significant BP fall were classified as severe CAN as per Ewings et al.

Observations and Data Analysis

Sex Distribution

Sex	Frequency	% of Study Group
Male	30	35.7
Female	54	64.3
Total	84	100

CAN Positivity

CAN	Aniscope		Conventional	
	Frequency	%	Frequency	%
Yes	70	83.3	75	89.3
No	14	16.7	9	10.7
Total	84	100	84	100

Severity of CAN

	Aniscope		Conventional	
	Frequency	%	Frequency	%
Early	9	12.8	19	25.0
Late	47	67.1	53	69.7
Advanced	14	20	4	5.26
Total	70	100	76	100

Valsalva Ratio

	Frequency	Percentage
Normal	16	19
Borderline	45	53.6
Abnormal	23	27.4
Total	84	100

E:I Ratio

	Frequency	Percentage
Normal	9	10.7
Borderline	15	17.9
Abnormal	60	71.4
Total	84	100

30:15 Ratio

	Frequency	Percentage
Normal	11	13.1
Borderline	20	23.8
Abnormal	53	63.1
Total	84	100

Orthostatic Hypotension

	Frequency	Percentage
Normal	59	70.2
Borderline	21	25.0
Abnormal	4	4.8
Total	84	100

Sensitivity and Specificity of the HRV Test

Test	Sensitivity (%)	Specificity (%)
Valsalva Ratio	86.7	66.7
E:I Ratio	92	33.3
30:15 Ratio	92	55.6

Association of Various Parameters with CAN

Parameter	% of Subjects	Prevalence of CAN		P Value	
		Conventional	Ansiscopes	Conventional	Ansiscopes
Smokers	15.5%	100%	92.3%	0.384	0.58
Hypertension	42.9%	91.9%	86.1%	0.799	0.761
Peripheral Neuropathy	32.1%	96.3%	92.6%	0.293	0.210
Duration of Diabetes					
a. At Diagnosis	19%	81.2%	68.8%	0.344	0.106
b. 1 - 5 years	59.5%	88%	82%		
c. > 5 years	21.5%	100%	100%		
Unaware	75%	93.7%	85.7%	0.039	0.049
Poor Sugar Control	33.33%	100%	96.4%	0.026	0.029

RESULTS

1. Among the total 84 individuals studied, 64.3% were females and 35.7% were males.
2. A total of 15.5% (13 persons) were smokers and among them 92.3% (12 persons) and about 100% (13 people) were found to have cardiac autonomic neuropathy by ansiscopes and conventional methods respectively.
3. 27 subjects, i.e. 32% were found to have peripheral neuropathy by clinical testing and among them 92.6% (25 persons) tested positive for autonomic neuropathy by ansiscopes and 96.3% (26 persons) by conventional method.
4. 36 subjects (42.9%) of the study population were hypertensive and of them 31 people (86.1%) and 33 people (91.7%) tested positive for autonomic dysfunction by ansiscopes and conventional method respectively.
5. Of the 84 study subjects, 63 (75%) people were not aware of the complications related to diabetes. In them 54 subjects (85.7%) and 59 subjects (93.7%) tested positive for cardiac autonomic neuropathy by ansiscopes and conventional methods respectively. This association is found to be statistically significant.
6. A total of 16 subjects (19%) were examined at diagnosis. Among them 11 individuals 68.8% were CAN positive by ansiscopes and 81.2% (13 subjects) by conventional method.
7. Of the 84 individuals 50 individuals were diabetic for a duration 1 - 5 years. Among them, 82% (41 individuals) and 88% (44 subjects) tested positive for autonomic dysfunction by ansiscopes and conventional method respectively.
8. Of the 18 subjects who were diabetic for more than 5 years were tested 100% positive for autonomic dysfunction by both the methods.
9. 28 individuals had poor glycaemic control. All individuals with poor glycaemic control tested positive for cardiac autonomic dysfunction by conventional method and 96.4% (27 individuals) tested positive by ansiscopes. This association is statistically significant.

Pathogenesis

Free radicals - a free radical is a species which has one or more unpaired free electron in its orbit.

Mechanism of Generation of Oxygen Free Radicals

Electron transfer reactions like hydroxyl radicals, superoxide anion radical, lipid alkoxyl and peroxy radical and hydrogen peroxide generate free radicals.

Energy transfer reactions like triplet carbonyl compounds and singlet oxygen are also involved in generation of free radicals.

Hyperglycaemia-Induced Mitochondrial Superoxide Production

During electron transfer in respiratory chain, a proton gradient is created by extrusion of protons into intermembrane space of mitochondria.

This gradient stimulates ATP synthase. In diabetes with high intracellular glucose concentration, more glucose is oxidised via citric acid cycle and electron donors like NADH, FADH₂ gets used up. After a critical threshold, the electron transfer inside complex 3 gets blocked. This process produces electron to get backed-up to coenzyme-Q, which donates electron to oxygen producing superoxide anion.

Hyperglycaemia activates other pathways like redox changes, NADPH oxidases and uncoupled eNOS gets amplified and produce superoxide.

Hyperglycaemia-Induced Mitochondrial Superoxide Production - Activates other Pathways by Inhibiting GAPDH⁽⁵⁾⁽⁶⁾

Intracellular hyperglycaemia reduces the glycolytic enzyme GAPDH. This causes other glycolytic intermediates to increase.

1. High levels of glyceraldehyde 3 phosphate - glycolytic metabolite activates following two pathways.
 - a. AGE pathway - glyceraldehyde 3 phosphate is the source for AGE precursor methylglyoxal.
 - b. Classic protein kinase C (PKC) pathway: glyceraldehyde 3 phosphate is the source for diacylglycerol, which is the activator of PKC pathway.
2. Levels of fructose 6 phosphate increase, which is a glycolytic metabolite activates hexose amine pathway to form UDP-GlcNAc.
3. GADPH inhibition also increases the intracellular glucose level, which enters polyol pathway.
 - a. Sorbitol is formed from glucose by the enzyme aldolase reductase consuming NADPH. Increased sorbitol is neurotoxic causing Schwann cell damage by increasing cell osmolarity.
 - b. Depletion of NADPH in the above process decreases intracellular myoinositol, which interfere with cellular metabolism.

Mechanism of Hyperglycaemia-Induced Cellular Damage

Cellular Injury due to Increased Reactive Oxygen Species

1. Oxygen free radicals attack the iron-sulphur moiety of enzymes and proteins and inhibit them. The proteins more susceptible for inhibition are complexes I - III of electron transfer chain, biotin synthase and aconitase of citric acid cycle.
2. Lipids in membranes of mitochondria, plasma and endoplasmic reticulum undergoes peroxidation. The end products of this process - lipid peroxides are toxic to the cell.
3. Proteins and nucleic acid in the cells undergo peroxidation and nitrosylation, which are toxic to the cell.
4. Oxidative modification of various transcription factor causes reduced expression of anti-apoptotic proteins like Bcl-2 and increase in pro-apoptotic proteins.
5. Oxidative damage of DNA, especially in non-dividing cells like neurons affect axonal transport and signalling resulting in loss of function of neurons.

NRF2 and Oxidative Stress

NRF2 is a transcription factor that protects against oxidative stress. NRF2 expression is down regulated in diabetic nerves. DRG neurons are protected from free radical injury via NRF2 activation. Hence, hyperglycaemia-induced downregulation of NRF2 makes Schwann cells and DRG more susceptible to oxidative stress.

Role of Advanced Glycation End Products in the Pathogenesis of Diabetic Neuropathy

AGEs

AGE are heterogeneous compounds, which are formed by non-enzymatic glycation and oxidation of proteins and/or lipids with aldose sugars.

Formation of AGE

Aldehyde of glucose combines with amino acid side chain and forms covalent bond, which is labile. Early glycation produces Schiff basis, which are reversible. These undergo further glycation and Amadori rearrangement to produce irreversible AGE products.

Examples

Pentosidine, Glycated HbA1C, N-carboxymethyl lysine, Methylglyoxal.

Formation of Advanced Glycation End Products

Receptor for Age (RAGE)

RAGE belongs to immunoglobulin receptor super family. They are expressed minimally in normal non-diabetic tissue and vessels. Chronic hyperglycaemia produces more AGE products, which by feedback mechanism upregulates RAGE expression. RAGE stimulation produces pro-inflammatory response.

AGE on Function of Extracellular Tissue

1. AGE produces cross-linking of elastin and type I collagen, thereby producing increased stiffness of vessels.
2. AGE interaction via RAGE decreases binding heparin sulfate, a proteoglycan in the vessel wall to the basement membrane producing a procoagulant state.
3. Glycation of LDL decreases nitrous oxide, thereby decreasing vasodilatation.
4. Glycated LDL decreases clearance and uptake of LDL producing a pro-atherogenic state promoting smooth muscle cell proliferation and atheroma formation in endoneurial vessels.

AGE on Function of Intracellular Milieu

1. Glycation of FGF affects vascular homeostasis.
2. AGE-induced stimulation of monocytes and endothelial cells causes increased expression of E-selectin, VEGF, VCAM-1, ICAM-1, other pro-inflammatory cytokines like IL-1, IL-6, TNF- α , RAGE.

Role of AGE in Diabetic Neuropathy⁽⁷⁾⁽⁸⁾

1. AGE/RAGE induce oxidative stress that increases glycosidation products like pentosidine.
2. Upregulation of nuclear product NF - kappa B and various pro-inflammatory genes alter neurological function.
3. Atherosclerotic endoneurial vessels produces ischaemic nerve damage.
4. Hyperglycaemia-induced AGE causes segmental demyelination of peripheral nerves.
5. AGE alters cytoskeletal proteins of axons like tubulin, actin, neurofilament and produces atrophy of axons and degeneration.
6. Glycation of laminin produces reduced axonal regeneration.
7. Endoneurial deficiency of nitrous oxide affects microvasculature.

Polyol Pathway

Peripheral nerves uptakes glucose in a non-insulin dependant manner. In hyperglycaemia, high glucose in the nerves enter polyol pathway.

Two Enzymes are involved in the above Pathway

- a. Aldose Reductase (AR) - in the presence of cofactor NADPH reduces glucose to sorbitol.
- b. Sorbitol Dehydrogenase (SDH) - in the presence of cofactor NAD⁺ forms fructose from sorbitol.
 1. Sorbitol oxidation by SDH produces NADH from NAD⁺. NADH acts as a substrate for NADH oxidase to produce ROS.
 2. Fructose formed is converted to 3 deoxyglucosone and fructose 3 phosphate. These are extremely potent non-enzymatic glycation agents. AGE acts via RAGE causing oxidative stress. Protein kinase C pathway.

Hyperglycaemia increases diacylglycerol, which activates PKC. Active PKC increases expression of TGF- β and other pro-inflammatory cytokines, which produces oxidative damage of the diabetic nerves.⁽⁸⁾

Hexosamine Pathway

Fructose 6-phosphate is converted to glucosamine 6-phosphate by hexosamine.

CONCLUSION

The prevalence of cardiac autonomic neuropathy is extremely high among diabetics as shown in our study. Most diabetic patients have significant autonomic dysreflexia even at diagnosis of diabetes. Cardiac autonomic neuropathy is the single important factor, which predicts the 5-year mortality rate among diabetics. Poor blood sugar control is significantly associated with CAN and is the single best factor, which determines the rate of progression as cited in our study. Early screening, early diagnosis, proper education of patients and

strict glycaemic control help in the arrest of progression of Cardiac Autonomic Neuropathy in Type 2 diabetic population.

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