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Can Proinflammatory Markers like IL6 and TNF Alpha be the early Predictors of Subclinical Peripheral Neuropathy in Prediabetes?

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ABSTRACT

Introduction: Polyneuropathy is the commonestcomplication present in almosthalf of the diabetic patients which is related directly with the duration and severity of hyperglycemia. Neuropathy can develop as of in the pre-diabetic phase, as evident from the different studies. The reason for this is the endothelial injury leading to micro inflammation.

Aim and Objectives: We extend our analysis to study the proinflammatory markers IL6 and TNF alpha in the subclinical peripheral neuropathy in prediabetes as biomarkers for the early risk predictors and therapeutic targets for prediabetes and related complications and to study the peripheral neuropathy in prediabetes.

Methods: A cross sectional study which will be carried in the medicine department at Acharya Vinoba Bhave Rural hospital at Sawangi.The individuals will be screened for prediabetes andaccording to American Diabetic Association i.e. Impaired fasting glucose-100 -125 mg/dl And/ OR Impaired glucose tolreance-140 - 199 mg/dl. Or HbA1C of5.7% -6.4% the subjects will be included in the study. Their serum TNF-α and IL-6 levels will be measured by BD CBA Flex capture beads method (B D BioSciences 2350 Qume Drive San Jose,CA 95131.Nerve Conduction Studieswill be performed using the standardized technique on bilateral tibial and sural nerves in lower limbs with surface recording.

Results: The proinflammatory markers will be raised in the patient who will have subclinical peripheral neuropathy than those who do not have neuropathy in prediabetic subjects.

Conclusion: The study will probably assist us to associate the relation of neuropathy with prediabetes and early intervention of treatment of prediabetes with the help of drugs rather than lifestyle modification.

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Keywords: prediabetes, neuropathy, inflammatory markers

INTRODUCTION:

Diabetic polyneuropathy(DPN) is the commonest clinical neuropathy developing in almost half of all diabetes patients which is related directly with the duration and severity of hyperglycemia. (1)It is estimated that two third of the diabetic patients have subclinical peripheral neuropathy. Subclinical neuropathy can be diagnosed by electrodiagnostic testing of peripheral nerves or by quantitative sensory testing.It is also seen that at the time of diagnosis of diabetes mellitus 18% of patients already have symptoms of pain in their legs which raises a question whether neuropathy develops at the prediabetic stage?

Neuropathy can develop as of in the pre-diabetic phase is evident from the different studies. (2,3,4)While answering the above question, it's exciting to look at the relationship between peripheral neuropathy and prediabetes.Prediabetes includes impaired glucose tolerance test and impaired fasting glucose (5) Therelation of peripheral neuropathy and prediabetes is important for two reasons; it will raise a question on the how the diagnosis of diabetes should be made and should we lower the blood glucose values to diagnose diabetes mellitus and secondly, it would be reasonable to treat prediabetes with medications instead the lifestyle change approach alone.

Endothelial injury leading to microinflammation is the reason behind late complications in diabetes mellitus is already proven. Various biochemical signals regulate the inflammation both locally and systemically. One amongst the signal is the cytokines molecules. Cytokines which is generally anti-inflammatory or proinflammatory are immune sensitive .The increase or the decrease in the factors is regulated by the immune response of the patient.In type 2 diabetes mellitus predominantly proinflammatory cytokines rises as compared to the anti-inflammatory cytokines which potentially leads to the increase in the complications of the diabetes mellitus. The association of proinflammatory factors as a marker of subclinical inflammation starts early before the onset and progression of distal peripheral neuropathy in the diabetes mellitus is clearly indicated in large prospective KORA) F4/FF4 cohort, study.(6) We extend our analysis to study the proinflammatory markers IL6 andTNF alpha in the subclinical peripheral neuropathy in prediabetes as biomarkers for the early risk predictors and therapeutic targets for prediabetes and related complications.

RATIONALE

Subclinical peripheral neuropathy is been seen in the prediabetes which is diagnosed by the electrodiagnostic study that is nerve conduction studies. This being the interventional, time consuming investigation requiring expert to carry and interpret the results we propose to study the serum levels of proinflammatory markers like IL6 and TNF alpha which can predict the early risk to stratify the complications of prediabetes.

RESEARCH QUESTION

Can proinflammatory markers Interleukin 6 (IL6) and Tumor necrosis factor(TNF) alpha be the early biomarkers to predict the neuropathy or any other complications in the prediabetes as that of the diabetes mellitus?

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Aim

To study the levels of proinflammatory markers like IL6 and TNF alpha in prediabetes with or without peripheral neuropathy.

OBJECTIVES

- To study peripheral neuropathy in prediabetes.
- To study other risk factors leading to peripheral neuropathy in prediabetes
- To correlate the cytokines and interleukins with different grades of peripheral neuropathy.

MATERIALS AND METHODS

Study design:It will be a Cross sectional study.

Study setting:The study will be carried in the medicinedepartment at JNMC Sawangi at AVBR Hospital in Wardha district.

Study population: The individuals will be screened for prediabetesaccording to American Diabetic Association i.e. Impaired fasting glucose-100 - 125 mg/dl And/ OR Impaired glucose tolerance-140 - 199 mg/dl. Or HbA1C of 5.7% -6.4% will be included in the study.In the prediabetes the following patients will be excluded.

Exclusion criteria

- Patients who deny participating in the study.
- Patients consuming alcohol
- Chronic Kidney Disease patients
- Patients havinghypothyroidism or hyperthyroidism.
- Diagnosed diabetes mellitus (On Insulin Or oral hypoglycemic drugs or life style management)
- Patients having macrocytic hypochromic anemia MCV>90
- Patients receivingAnti-convulsant treatment, Anti-Retroviral and Anti tubercular Treatment.
- Patients diagnosed to have malignancy.
-

Time frame: The study will be carried out for a period of 3 years.

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PICO format

Population	**Patients of Prediabetes with peripheral neuropathy**
Intervention	Measurement of TNF alpha and IL 1,6
comparison	Patients of Prediabetes without peripheral neuropathy
outcome	Early predictors of subclinical peripheral neuropathy in prediabetes
Time	3 years

METHODOLOGY:

After getting the approval from the Institutional Ethics committee the study will be carried. The individuals attending the medicine outpatient department will be screened for prediabetes after taking the written consent for their participation in the study.Those diagnosed to have prediabetes as per ADA criteria will be enrolled in the study. Demographic data like height, weight and waist circumference will be recordedand Body mass index will be calculated.Their serum TNF-α and IL-6 levels will be measured by BD CBA Flex capture beads method (B D BioSciences 2350 Qume Drive San Jose,CA 95131. Lipid profile will be determined by the enzyme chemical method.

By using the standard technique **nerve Conduction Studies** will be conducted on bilateral sural and tibial nervesin lower extremities for sensory neuropathy andmotor neuropathy respectively. The following data will be obtained from the electrophysiological graph of the nerves:

- CMAP (Compound muscle action potential)
- SNAP (Sensory nerve action potential)
- Nerve conduction velocity (NCV).

For sural nerve,nerve conduction will be recorded byplacing the surface electrode between tendoachilles and lateral malleolus. Antidromically stimulation will be given proximally10 - 16 cm to the recording electrode, distally to lower border of gastrocnemius at the lower one third of the leg. Conduction velocity if less than 40 m/s and amplitude of SNAP is less than 7 µV for sural nerve will be considered abnormal.

Tibial nerve conduction will be done by placing the active surface electrode on the abductor hallucis muscle slightly below and anterior to navicular tuberosity. Stimulation will be given to the medial malleolus behind and proximally and the popliteal fossa along the flexor crease mildly lateral to the midline of the popliteal fossa. Conduction velocity for tibial nerve if is lesser than 50 m/s and amplitude of CMAP ifless than 8 µV will be considered abnormal.

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The trained physician will interpret the electrophysiological graphs. Sensory neuropathy will be labeled if any one or more of thebelow findings will be present in sural nerve:

- Reduced SNAP amplitude (< 7 µV)
- Decreased conduction velocity (< 40 m/s).

In tibial nerve, motor neuropathy will be labeled if any one or more of the findings will be present

- Reduced CMAP amplitude (< 8 µV)
- Decreased conduction velocity (< 50 m/s).

Statistical Methods:

- Data will be analyzed using STATA 12 software
- Numerical data will be summarized using means and standard deviations.
- The baseline measures will be compared using the paired-samples, two-sided *t*-test.
- Comparisons between the two groups with respect to numeric variables will be done by the Student's t test for parametric data and Mann-Whitney test for non-parametric data. All p-values <0.05 will be considered significant.
- Correlation between the variables will be decided by Pearson's correlation analysis and logistic regression analysis will beby multivariate analysis.

Statistical Software: STATA software

Sample size calculation

Hypothesis testing for two mean (equal variance)

- Standard deviation in group 1= 4.64
- Standard deviation in group 2 = 4.78
- Effect size = 0.488322717622081
- Power(%) = 95
- Alpha Error (%) = 5
- sided = 2

Required sample size = 109

Study Variable:

The following variables will becollected in the data sheet

- Body mass index
- Blood Sugar level
- TNF-α and IL-6 level
- Nerve conduction study parameter
- Lipid profile

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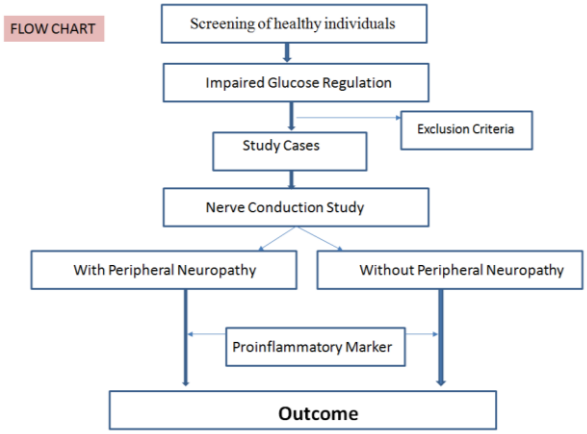
Outcome measures:

The primary outcome is to measure the levelsof serum IL6 and TNF alpha in the prediabetes with or without subclinical peripheral neuropathy. The secondary outcome is to see the prevalence of prediabetes.

EXPECTED RESULT:

The proinflammatory markers will be raised in the patient who will have subclinical peripheral neuropathy than those who do not have neuropathy.



DISCUSSION:

Prediabetes is an intermediate zone or a grey zone which lies between normoglycemia and full blown diabetes mellitus. Besides, landing to overt diabetes prediabetes is also associated with microvascular as well macrovascular complications as evident from the literature. With this the complications like neuropathy associated with prediabetes is also emerging problem. From India, in relation to prediabetic neuropathy there is paucity of literature.The nerve conduction studies (NCS) are electro- diagnostic tests which are used to assess the ability of the electrical conduction of the motor and sensory nerves. Studies found that the changes in the nerve conduction amplitude and velocity is mainly associated with diabetic peripheral neuropathy. Accumulating evidence suggests that half of patients with type 2 diabetes mellitus (T2DM) will eventually experience neuropathy and progressive injury of nerve fibers during the course of diabetes, and severe neuropathic symptoms result in poor quality of life.

Kannan et al in 2014, detected neuropathy in 32.8% patients of prediabetes.(7) The study was case control .It was conducted on 30 subjects who were age matched controls and 58 of impaired oral glucose tolerance test (OGTT. Nerve conduction studies (NCS) was performed on one upper and both lower extremities. Neuropathy was evaluated on sural nerve, planter nerve (medial and lateral) by using standard techniques. The author also found that those with family history of diabetes were at risk of developing prediabetic neuropathy. High prevalence of prediabetic neuropathy was also found in individuals with dyslipidemia and BMI of <30.

Lee et al in 2015 found 49%prevalence of neuropathy in prediabetes.(8)It was a cohort of 467 individuals in the longitudinal PROMISE (Prospective Metabolism and Islet Cell

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Evaluation) .Peripheral neuropathy was evaluated by Michigan Neuropathy Screening Instrument (MNSI) scores (>2), and the vibration perception thresholds (VPTs) was used to measure the severity of nerve dysfunction by using a neurothesiometer.Clinical neuropathy may be present before the onset of diabetes .They also added that the risk for neuropathy increases if the risk factors for diabetes onset are present.

Hyperglycemia, leading to endothelial injury and causing inflammation by the release of proinflammatory factors is well known fact in diabetes mellitus. This is the main culprit for the micro and macro vascular complications in diabetes mellitus. Probably the same mechanism also applies to the prediabetes. Studying this proinflammatory factors in the prediabetes patients would predict the early development of complications.

T Duksal et al(9) studied nerve conduction studies (NCS) of the sensory nerves of dorsal sural and medial plantar and found abnormalities were present inprediabetes and type 2 DM than the control. They also concluded that patients of diabetes mellitus have significantly higher levels of TNF-αlevels and in prediabetes and diabetes mellitus group both have significantly lower levels of IL-10. They also concluded that in both the prediabetes and diabetes mellitus there is no correlation between the levels of HbA1c, TNF-α, IL-10, and abnormalities in NCS of the nerves.

Brahimaj A et al (10)found higher levels of EN-RAGE(S100A12 or Calgranulin C), a pro inflammatory protein which binds calciumand is secreted by granulocytes, in both the prediabetes and diabetes mellitus. Interleukin 13, a cytokine produced by the T-helper cells, mast cells, dendritic cells, basophils , eosinophils and keratinocyteswas associated with lower risk of prediabetes.

An Italian cross-sectional study (11)found that lower RAGE plasma levels was found in prediabetic patients but increased levels of S100A12,a proinflammatory factor, was found in both prediabetic and diabetic patients.

Grossman V et al(12) in a large sample of 15,010 subjects demonstrated thatwith the development and progression of type 2 diabetes there is a variation of the inflammatory and immune biomarkers. Varying dynamics of the biomarkers of inflammation and immunity was seen with the advancing disease of the diabetes mellitus, enabling differentiation of the early preclinical and prediabetes, its complications, and disease progression by intensity of medical treatment.

Tiftikcioglu et al(13)found that the prediabetics have significantly higher levels of HbA1c and IL-6 than the controls. In patients with prediabetes, IL-6 levels were significantly correlated with levels of CRP, fibrinogen, ESR and sE-selectin. Prediabetic patients had changes in very distal sensory nerves. In prediabetic patients,IL-6 levels were positively correlated with HbA1c and negatively correlated with neuropathy.

The increasing trend of prediabetes and its complications(14) compels the researchers to focus more on its prevention and target specific treatment like targeting the inflammatory pathways. Also the understanding of the inflammatory mechanisms in the prediabetes should

be improved as this would strategify the prevention and the control of prediabetes and curbing the path towards diabetes mellitus.

CONCLUSION:

The study will probably help us to associate the relation of neuropathy with prediabetes and early intervention of treatment of prediabetes with the help of drugs rather than lifestyle modification.

REFERENCES:

[1] International Diabetes Federation (IDF) 2017. IDF Diabetes Atlas 8th ed.

[2] Tesfaye S, Selvarajah D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. Diabetes Metab Res Rev. 2012; 28(Suppl 1):8–14.

[3] Callaghan BC, Cheng HT, Stables CL, Andrea L, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. Lancet Neurol. 2012; 11:521–34.

[4] Papanas N, Ziegler D. Prediabetic neuropathy: does it exist? Curr Diabetes Rep. 2012;12:376–83.

[5] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37 Suppl 1:S81–S90.

[6] Herder C, Kannenberg JM, Huth C, Carstensen-Kirberg M, Rathmann W, Koenig W, Heier M, Püttgen S, Thorand B, Peters A, Roden M, Meisinger C, Ziegler D. Proinflammatory Cytokines Predict the Incidence and Progression of Distal Sensorimotor Polyneuropathy: KORA F4/FF4 Study. Diabetes Care. 2017; 40(4):569-576.

[7] Kannan MA, Sarva S, Kandadai RM, Paturi VR, Jabeen SA, Borgohain R. Prevalence of neuropathy in patients with impaired glucose tolerance using various electrophysiological tests. Neurol India. 2014;62(6):656-61.

[8] Lee CC et al. Peripheral neuropathy and nerve dysfunction in individuals at high risk for type 2 diabetes: The PROMISE cohort. *Diabetes Care* 2015; 38:793

[9] Duksal T, Tiftikcioglu BI, Bilgin S, Kose S, Zorlu Y. Role of inflammation in sensory neuropathy in prediabetes or diabetes. Acta Neurol Scand. 2016 May;133(5):384-90.

[10] Brahimaj A, Ligthart S, Ghanbari M, et al. Novel inflammatory markers for incident pre-diabetes and type 2 diabetes: the Rotterdam Study. *Eur J Epidemiol*. 2017;32(3):217-226.

[11] Di Pino A, Currenti W, Urbano F, et al. Low advanced glycation end product diet improves the lipid and inflammatory profiles of prediabetic subjects. J Clin Lipidol. 2016;10(5):1098–1108.

[12] Grossmann V, Schmitt VH, Zeller T, et al. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. Diabetes Care. 2015;38(7):1356–1364.

[13] Tiftikcioglu BI, Duksal T, Bilgin S, Kose S, Zorlu Y. Association between the Levels of IL-6, sE-Selectin and Distal Sensory Nerve Conduction Studies in Patients with Prediabetes. Eur Neurol. 2016;75(3-4):124-31.

[14] Rathi, N., B. Taksande, and S. Kumar. "Nerve Conduction Studies of Peripheral Motor and Sensory Nerves in the Subjects with Prediabetes." Journal of Endocrinology and Metabolism 9, no. 5 (2019): 147–50. https://doi.org/10.14740/jem602.