ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

An analytic cross-sectional study to explore the role of TNF-α level as a marker for diabetic polyneuropathy

Debarati Chanda[1], Aditi Aikat[2], Saswati Ray[3], Sangita Sen[4], Debjani Chakraborti[5]

[1]Associate Professor, [3]Assistant Professor, [5]Professor, Department of Physiology, [2]Professor, Department of Community Medicine, Jagannath Gupta Institute of Medical Sciences and Hospital, Kolkata, West Bengal, India, [4]Professor, Department of Physiology, IPGME and R, Kolkata, West Bengal, India

Submission: 14-03-2022 Revision: 29-05-2022 Publication: 01-07-2022

ABSTRACT

Background: Diabetes has reached epidemic proportion in India. The most common complication of Type 2 diabetes mellitus (T2DM) is diabetic polyneuropathy (DPN). Various theories have been proposed to explain development of DPN, among which newer concepts are activation of inflammatory pathways. **Aims and Objectives:** The aim of the study was to determine the relationship between tumor necrosis factor alpha (TNF-α) levels and painless and painful variants of DPN in T2DM patients. **Materials and Methods:** The study was carried out on 57 subjects. The subjects were divided into four groups; Group 1 had 15 healthy controls without diabetes; Group 2 had 12 control group patients of T2DM without neuropathy; 20 patients of T2DM with painful neuropathy were included in Group 3 and 10 T2DM patients with painless neuropathy. TNF-α level was measured by drawing 5 ml blood from individual patients using Ray Bio Human TNF-α enzyme-linked immunoassay kit. **Results:** Plasma TNF-α level increases from non-diabetic control (221.7 ± 46.91 pg/ml) to diabetic control (743.6 ± 87.27 pg/ml) and also further increased in painful neuropathy patients (1343 ± 283.8 pg/ml). Interestingly, TNF-α was found to be significantly decreased (441.4 ± 118.3 pg/ml) in patients with painless neuropathy. **Conclusion:** TNF-α level increased in diabetic neuropathy but did not correlate with severity of neuropathy.

Key words: Diabetic polyneuropathy; Painful neuropathy; Painless neuropathy; Tumour necrosis factor alpha; Type 2 diabetes mellitus

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v13i7.43808

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The history of diabetes is as old as the history of medicine. However, nowadays, diabetes mellitus (DM) is a very serious and ever-growing health issue globally. It has reached epidemic proportion in India.[1] Between the two types of diabetes, the most common variant is Type 2 DM (T2DM).[2] Diabetic polyneuropathy (DPN) is one of the most common complications of T2DM.[3] The pathogenesis of DPN is still a matter of debate. Hence, various theories have been proposed to explain development of DPN, among which newer concepts are activation of inflammatory pathways[4] and increase in cytokines like tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6).[5] TNF-α is a pleiotropic cytokine and is essential for homeostasis of peripheral nervous system.[6] Clemente-Gonzalez found increased TNF-α level in diabetic neuropathy regardless of glycemic control.[7] Baka et al., suggested that a proinflammatory stage is commonly associated with pain in DPN patients.[8] Some researchers[9-11] showed that inflammatory cytokine level is significantly less in painless neuropathy compared to painful neuropathy.

The role of TNF-α in development of DPN has been studied and evaluated in several ways in different population. However, data are limited for our Indian population. Therefore, our study aimed to determine the relationship between TNF-α levels and the variants of DPN in T2DM patients and explore the proclaimed role of TNF-α as a marker for DPN among the Indian.

Address for Correspondence:
Dr. Debarati Chanda, Associate Professor, Department of Physiology, Jagannath Gupta Institute of Medical Sciences and Hospital, 42B James Long Sarani, Kolkata - 700034, West Bengal, India. **Mobile:** +91-9830775956. **E-mail:** mukherjeedebarati9@gmail.com

Aims and objectives

To determine the relationship between TNF α (Tumour Necrosis Factor Alpha) levels and painless and painful variants of diabetic polyneuropathy (DPN) in T2DM patients.

MATERIALS AND METHODS

It is a cross-sectional observational study conducted in the Physiology Department of IPGMER in association with endocrinology department over a year from June 2019 to May 2020. In our study, we have taken patients attending the outpatient department of Endocrinology Department of IPGMER, diagnosed with T2DM in the age group of 30–60 years. Institutional Ethical Committee clearance (Inst/IEC/1307) was obtained and due informed consent taken from eligible study subjects. Patients having evidence of neuropathy from history, clinical examination, vibration perception threshold (VPT), and nerve conduction study (NCS) were included in the study, while, patients having neuropathy because of some other causes including nutritional deficiency, chemotherapy, hypothyroidism were excluded from the study. We also excluded patients with malignancy, pregnancy, and autoimmune disease.

Sample size calculation

The sample size was calculated using effect size. Effect size is standard measure of the difference between two or more groups independent of the unit of the outcome variable. To make it simple, effect size is categorized into three levels-small, medium, and large. Keeping alpha probability of 0.05 and power of 0.80, in a one-way analysis of variance (ANOVA) with fixed effects single factor design, the calculated values of the effect size at small, medium and large levels are 0.1, 0.25, and 0.4, respectively.[12] Based on clinical judgment and practical feasibility, we decided to use a large effect size (0.6) in our study. The total sample size for the specified effect size was calculated as 54 for four independent groups. Thus, 14 patients were required per group for the study. The sample size estimation was carried out using G*power version 3.1.9.2. During the recruitment of patients while conducting the study, because of practical difficulties and non-availability of patients in the respective groups, we could recruit 15, 12, 20, and 10 patients in the Groups I, II, III, and IV, respectively. We have mentioned this shortcoming of our study in the limitations section.

The study was carried out on 57 subjects during the study period, based on convenience sampling. The subjects were divided into four groups; Group 1 had 15 healthy controls without diabetes; Group 2 had 12 control group patients of T2DM without neuropathy; 20 patients of T2DM with painful neuropathy were included in Group 3, and 10 T2DM patients with painless neuropathy were included in Group 4. Detailed histories were taken regarding age, duration of diabetes, body mass index, and HbA1c and severity of polyneuropathy was assessed by VPT and NCS. The VPT was measured at the great toe on the dominant side of each patient using a biothesiometer. A VPT >25 volts was considered to be abnormal.[13]

In the NCS, each case underwent neuroelectrophysiological examination of the peripheral nervous system using instrument for neuroelectrophysiology tests' RMS NCV EMG EP MARK II with Aleron201 Electromyograph with RMS Stimulator.

Parameter	MNC	SNC
Gain	2mV/div	20μV/div
Time base	5ms/div	2ms/div
Low frequency filter	20Hz	2Hz
High frequency filter	10KHz	2KHz
Stimulus frequency	1Hz	1Hz
Stimulus duration	Depends on particular patient	
Stimulus intensity	Depends on particular patient	

MNC – Motor Nerve Conduction SNC- Sensory Nerve Conduction

ronto Clinical Neuropathy Score, sensory, symptoms, and reflex scores were assessed to assign the study subject as having polyneuropathy or not. Pin sensitivity, vibration sensitivity, muscle power, reflex, sensory nerve action potential, and motor nerve action potential were assessed.

The patients were diagnosed to have neuropathy (painful and painless) by neuropathy symptom score.[14] For the evaluation of the NSS, the participants were asked about the following symptoms in their feet or legs: (1) Pins and needles; (2) abnormal cold or hot sensations; (3) lancinating pain; (4) deep aching pain; (5) burning pain (causalgia); and (6) irritation of the feet or legs by the bedclothes at night (hyperesthesia). Each symptom was scored with 1 point if it was present and 2 points if nocturnal exacerbation was also present. A score of 4 or more points was considered to be abnormal.

TNF-α level was measured by drawing 5 ml blood from individual patients. We centrifuged and separated the plasma and TNF-α level was measured with Human TNF-α enzyme-linked immunoassay (ELISA) kit (Ray Bio Human IL6 ELISA kit).[15]

After collection of all data, compilation and subsequent analysis was carried out using appropriate statistical tests. Calculation of mean±SD was done for each measurement. One-way ANOVA and Student's t-test were performed and statistical analysis was carried out by using the GRAPHPAD PRISM Version 5.00 March 7, 2007.

RESULTS

Participants

Group 1: Healthy controls without diabetes; Group 2: Control group patients of T2DM without neuropathy; Group 3: T2DM with painful neuropathy, and Group 4: T2DM patients with painless neuropathy.

Descriptive data

Mean age of patients of Group 4 is higher than that of Group 1, 2, and 3. Duration of diabetes in Group 4 is also much higher than Group 1, 2, and 3. HbA1c level also increased through the groups and Group 4 showed poorest glycemic control. When we assess the neuropathy score, Group 4 showed significantly higher neuropathy score than Group 3. Plasma TNF-α level increases through Group 1 to Group 3 but interestingly, TNF-α was found to be significantly decreased in Group 4. Plasma TNF-α level did not correlate with severity of neuropathy.

Plasma TNF-α level was 221.7±46.91 pg/ml in Group 1, 743.6±87.27 in Group 2, and 1343±283.8 in Group 3. Hence, there was gradual increase of plasma TNF-α level and the P-value between Groups 1 and 3 and between Groups 2 and 3 was highly significant (P<0.0001 in both situations) in intergroup statistical analysis.

Plasma TNF level is 441.4±118.3 in Group 4 which was lower than Group 2 (743.6±87.27) and higher than Group 1 (221.7±46.91). Intergroup statistical analysis showed that there was moderately significant increase (P<0.01) between Group 1 and Group 4 and highly significant decrease (P<0.0001) between Group 2 and Group 4.

Plasma TNF level was 1343±283.8 pg/ml in Group 3 but in Group 4, the level drastically decreased to 441.4±118.3 pg/ml which is highly significant (P<0.0001) in intergroup statistical analysis.

DISCUSSION

Our study was conducted to establish any relationship between plasma TNF-α concentration and the presence of polyneuropathy, both painful and painless variants in T2DM patients of India and explore the possibility of TNF-α as a marker for the same. In our study, we found that there was gradual increase of plasma TNF-α level from Group 1 to Group 3 and the P value between Groups 1 and 3 and between Groups 2 and 3 was highly significant (P<0.0001 in both situations) in intergroup statistical analysis (Table 1).We also observed that though there is increase in plasma TNF-α in Group 1 and Group 2 but interesting finding was that TNF-α significantly decreased in Group 4 (vide Table 2). Intergroup statistical analysis showed there was moderately significant increase (P<0.01) between Group 1 and Group 4 and highly significant decrease (P<0.0001) between Group 2 and Group 4. Plasma TNF level was significantly decreased in Group 4 (P<0.0001) vide intergroup statistical analysis (Table 3). Plasma TNF-α level did not correlate with severity of neuropathy.

We suggest that pro-inflammatory marker TNF-α is significantly correlated with the severity of pain in diabetic neuropathy patients. In diabetic control group (Group 2), TNF-α level showed a definite tendency to rise that increases further with development of painful neuropathy (Group 3). However, in painless neuropathy (Group 4) although with high TNS score, there is a definite decrement in TNF-α marker. Hence, we can suggest that plasma TNF-α level did not correlate with severity of neuropathy.

Clemente-Gonzalez[7] found increased TNF-α level in diabetic neuropathy regardless of glycemic control. We also had similar observation. Galloway and Chattopadhyay[16] (2013) in his animal study with Zucker diabetic fatty rat (ZDF) found that there is an increase in inflammatory markers TNF-α, IL-1, 6, and 17 in the Dorsal Root Ganglion (DRG) of T2DM ZDF rat at the onset of pain in painful neuropathy suggest that inflammation in the DRG may play an important role in the development of pain in their model. Their finding is in accordance with our observation. Empl et al.,[9] in his study demonstrated that patient with painful neuropathies showed a stronger TNF-alpha immunoreactivity in myelinating Schwann cells compared with patients with non-painful neuropathy, similar to our observation. Mu et al.,[10] observed increase in plasma TNF-α levels in painful DPN patients compared to painless DPN patients. This observation resonates with our finding. Nurcan et al.,[11] in his study measured blood levels of pro-inflammatory cytokines (IL-1, 6, and TNF-α) and anti-inflammatory cytokines (IL-4 and 10) in 32 patients with painful DPN and 20 patients with painless DPN and also in 38 healthy controls. There was increase in pro-inflammatory cytokines in painful DPN group whereas anti-inflammatory cytokines were higher (2 fold) in painless DPN patients. He concluded that pro-inflammatory cytokine profile seems to be associated with pain in the setting of a peripheral neuropathy. Saleh et al.,[17] in his study found that reduced cytokine expression in DRG is related to diabetic sensory neuropathy so he inferred that diabetic neuropathy does not involve neuroinflammatory component. However, in our study, we found that in painful neuropathy group, inflammatory cytokines like TNF-α increases significantly and in painless neuropathy the TNF-α level in plasma decreased significantly. Hence, our result suggests involvement of

Table 1: Comparison of plasma TNF-α level between normal control, diabetic control, and painful neuropathy with statistical significance

Parameters	Group 1 Mean±SD	Group 2 Mean±SD	Group 3 Mean±SD	Stat significance (P-value) 1 versus 3	Stat sig (P-value) 2 versus 3
TNF-α (pg/ml)	221.7±46.91	743.6±87.27	1343±283.8	<0.0001 (s)	<0.0001 (s)

Table 2: Comparison of plasma TNF-α level between normal control, diabetic control, and painless neuropathy with statistical significance

Parameters	Group 1 Mean±SD	Group 2 Mean±SD	Group 4 Mean±SD	Stat sig 1 and 4 (P-value)	Stat sig 2 and 4 (P-value)
TNF-α (pg/ml)	221.7±46.91	743.6±87.27	441.4±118.3	<0.01 (s)	<0.001 (s)

Table 3: Comparison of plasma TNF-α between painful and painless neuropathy with statistical significance

Parameters	Group 3 Mean±SD	Group 4 Mean±SD	Stat sig (P-value)
TNF-α (pg/ml)	1343±283.8	441.4±118.3	<0.0001 (s)

inflammatory cytokines, contrary to their finding. Pallai Shillo[18] observed difference in inflammatory mediators between painful- from painless-neuropathies which supports our finding. The observations of Thomas Eko Purwata[19] also supports our finding.

Limitations of the study

Being single-centered and cross-sectional study carried out in a relatively small sample size, our study may not have been able to distinguish the other coincidental factors affecting the level of TNF-α.

CONCLUSION

Our study arrived at an interesting derivation that although TNF-α level correlated significantly with the progression of polyneuropathy in T2DM and presence of polyneuropathy, it showed a dip, in the painless polyneuropathy, while increased in painful polyneuropathy. Increasing levels of TNF-α were also noted with increasing severity of DPN. This is suggestive of the fact that the increasing levels of TNF-α may be taken as a marker for progression of T2DM and probability of having polyneuropathy and painful polyneuropathy, substantiating its role as a pro-inflammatory cytokine. However, the decreasing levels of TNF-α in painless DPN limit its role as a marker in this variant of DPN.

ACKNOWLEDGMENT

I express my sincere gratitude to Diabetic patients and Endocrinology department of IPGME&R.

REFERENCES

1. Menon VU, Kumar KV, Gilchrist A, Sugathan TN, Sundaram KR, Nair V, et al. Prevalence of known and undetected diabetes and associated risk factors in central kerala ADEPS. Diabetes Res Clin Pract. 2006;74(3):289-289.
https://doi.org/10.1016/j.diabres.2006.03.025
2. Kaveeshwar SA and Cornwall J. The current state of Diabetes Mellitus in India. Australas Med J. 2014;7(1):45-48.
https://doi.org/10.4066/AMJ.2013.1979
3. Jin HY and Park TS. Role of inflammatory biomarkers in diabetic peripheral neuropathy. J Diabetes Investig. 2018;9(5):1016-1018.
https://doi.org/10.1111/jdi.12794
4. Alvin CP. Harrisons Principles of Internal Medicine. 18th ed., Ch. 344. Diabetes Mellitus. New York: McGraw-Hill 2008. p. 2968-3002.
5. Zhou YQ, Liu Z, Liu ZH, Chen SP, Li M, Shahveranov A, et al. Interleukin-6: An emerging regulator of pathological pain. J Neuroinflammation. 2016;13(1):141.
https://doi.org/10.1186/s12974-016-0607-6
6. Raffaele S, Lombardi M, Verderio C and Fumagalli M. TNF Production and release from microglia via extracellular vesicles: Impact on brain functions. Cells. 2020;9(10):2145.
https://doi.org/10.3390/cells9102145
7. Clemente-Gonzalez JM, Mauricio D, Richart C, Broch M, Caixas A, Megia A, et al. Diabetic neuropathy is associated with activation of TNF alpha system in subjects with T1DM. Clin Endocrinol. 2005;63(5):525-529.
https://doi.org/10.1111/j.1365-2265.2005.02376.x
8. Baka P, Escolano-Lozano F and Birklein F. Systemic inflammatory biomarkers in painful diabetic neuropathy. J Diabetes Complications. 2021;35(10):108017.
https://doi.org/10.1016/j.jdiacomp.2021.108017
9. Empl M, Renaud S, Erne B, Fuhr P, Straube A, Wiemers NS, et al. TNF-alpha expression in painful and nonpainful neuropathies. Neurology. 2001;56(10):1371-1377.
https://doi.org/10.1212/wnl.56.10.1371.
10. Mu ZP, Wang YN, Li CQ, Lv WS, Wang B, Jing ZH, et al. Association between tumor necrosis factor-α and diabetic peripheral neuropathy in patients with Type 2 diabetes: A meta-analysis. Mol Neurobiol. 2017;54(2):983-996.
https://doi.org/10.1007/s12035-016-9702-z
11. Nurcan U, Rogausch P, Toyka KV and Sommer C. Differential expression of cytokines in painful and painless neuropathies. Neurology. 2007;69(1):142-149.
https://doi.org/10.1212/01.wnl.0000265062.92340.a5.

12. Ahmed SN, Jhaj R, Sadasivam B and Joshi R. Regression of the left ventricular hypertrophy in patients with essential hypertension on standard drug therapy. Discoveries (Craiova). 2020;8(3):e115.
https://doi.org/10.15190/d.2020.12
13. Phillip AL. In: Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ, editors. Joslin's Diabetes Mellitus. 14th ed. New York: Lippincott Williams and Wilkins; 2007. p. 823-970.
14. Doupis J, Lyons TE, Wu S, Gnardellis C, Dinh T and Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless neuropathy. J Clin End Metab. 2009;94(6):2157-2163.
https://doi.org/10.1210/jc.2008-2385
15. Human TNFα ELISA Kit. Available from: https://www.raybiotech.com [Last accessed 2021 May 05].
16. Galloway C and Chattopadhyay M. Increase in inflammatory mediators in DRG implicate in the pathogenesis of painful neuropathy in T2DM. Cytokine. 2013;63(1):1-5.
https://doi.org/10.1016/j.cyto.2013.04.009
17. Saleh A, Smith DR, Balakrishnan S, Dunn L, Martens C, Tweed CW, et al. Tumor necrosis factor-α elevates neurite outgrowth through an NF-κB-dependent pathway in cultured adult sensory neurons: Diminished expression in diabetes may contribute to sensory neuropathy. Brain Res. 2011;1423:87-95.
https://doi.org/10.1016/j.brainres.2011.09.029
18. Shillo P, Sloan G, Greig M, Hunt L, Selvarajah D, Elliot E, et al. Painful and painless diabetic neuropathies: What is the difference? Curr Diabetes Rep 2019;19(6):32.
https://doi.org/10.1007/s11892-019-1150-5
19. Purwata T. High TNF-alpha plasma levels and macrophages iNOS and TNF-alpha expression as risk factors for painful diabetic neuropathy. J Pain Res. 2011;4:169-175.
https://doi.org/10.2147/JPR.S21751

Authors Contribution:
DC- Conceptualized and also designed the study, literature search, interpretation, prepared first draft of manuscript; **SR-** Interpretation and literature search; **AA-** Conceptualized and critical revision of manuscript; **SS-** conceptualized and designed the study; and **DC-** Review of the study.

Work attributed to:
Endocrinology department, Physiology department of IPGME&R Kolkata.

Orcid ID:
Dr. Debarati Chanda - https://orcid.org/0000-0002-8990-2830
Dr. Aditi Aikat - https://orcid.org/0000-0001-5448-1557

Source of Support: Nil, **Conflict of Interest:** Declaration by all authors in accordance with ICMJE uniform disclosure form that no financial support was received, and none of them are presently or in the remote past three years, having any financial relationship with any organization having competing interest or with any organization that may influence the results of this study.