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Original Article

Exploring the Relationship between TNF-α Gene Expression in Non Diabetic Nephropathy Type 2 Diabetes Patients

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ABSTRACT

Non-diabetic nephropathy (NDN) is a common complication of type 2 diabetes, leading to kidney damage and impaired kidney function. TNF-α (tumor necrosis factor-alpha) is a pro-inflammatory cytokine that has been implicated in the development of NDN. **Objective:** To evaluate the gene expression of TNF-α in patients with type 2 diabetes mellitus (T2DM) without nephropathy to gain insight into the potential role of TNF-α in the pathogenesis of diabetic nephropathy (DN). **Methods**: Total of 80 subjects were tested, split into two groups, including healthy patients, T2DM patients without nephropathy, and T2DM patients with nephropathy. RNA was extracted from blood samples, and RT-PCR was used to observe the impact of T2DM without nephropathy on the expression of the TNF-α gene using gene-specific primers and SYBR Green mix. **Results:** The results showed almost 4.4-fold induced expression of TNF-α in T2DM patients without nephropathy compared to the normal group. **Conclusion:** The findings may have implications for the development of new therapies and biomarkers for DN, and for a good interpretation of the complex pathophysiology of T2DM. The exact role of TNF-α in the pathogenesis of DN in humans is not fully understood, and further investigation is needed.

INTRODUCTION

Hyperglycemia brought on by insulin resistance and/or inadequate insulin production characterizes diabetes mellitus, a chronic metabolic condition. With 90% of cases globally, type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes [1]. T2DM is linked to a number of microvascular and macrovascular consequences, including stroke, retinopathy, neuropathy, and nephropathy [2]. Around 30% of T2DM patients get nephropathy, one of the disease's most prevalent and harmful consequences [3]. Albuminuria, a decreasing glomerular filtration rate (GFR), and structural alterations in the kidney, such as mesangial enlargement, glomerular basement membrane thickening, and interstitial fibrosis, are all signs of the progressive kidney disease known as diabetic nephropathy (DN) [4]. Many variables, including genetic susceptibility, hyperglycemia, dyslipidemia, hypertension, and inflammation, have a role in the aetiology of DN [5]. Inflammation has a significant role in the pathophysiology of DN and other T2DM problems [6]. The pro-inflammatory cytokine tumour necrosis factor-alpha (TNF-alpha) is essential for controlling inflammation and immunity [7]. TNF- is generated by a variety of cells, including monocytes, macrophages, T cells, and adipocytes, and it interacts with a variety of cell types, including endothelial cells, mesangial cells, and podocytes, to cause tissue damage and inflammation [8]. The role played by TNF- in the onset and development of DN has been the subject of several investigations. It has been

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shown that TNF- expression is elevated in the kidney of DN patients as well as in DN animal models [9, 10]. By its impact on endothelial dysfunction, oxidative stress, and fibrosis, TNF- has been linked to the pathophysiology of albuminuria, GFR reduction, and structural abnormalities in the kidney [11, 12]. More research is required since it is unclear exactly how TNF-a contributes to the pathophysiology of DN in people. In order to better understand the possible function of TNF- in the aetiology of DN, we evaluated the gene expression of TNF- in patients with T2DM without nephropathy in this research. Our research may have ramifications for the discovery of novel DN treatments and biomarkers, as well as for a deeper understanding of the intricate pathophysiology of T2DM.

METHODS

It was a cross sectional case-control study. The research work was carried out in the Immunology department and Resource lab UHS, Lahore. The calculated sample size for each group is 40. A total of 80 subjects were tested for this study and they were divided into two groups of 40 individuals in each group. Group-I with 40 healthy patients. Group-II with 40 patients of T2DM patients without diabetic nephropathy. Five ml venous blood was collected in EDTA coated vacationers from T2DM patients with and without nephropathy and was brought to the Resource lab within four hours of the sample collection to avoid genomic RNA degradation. The primers were suspended using low TAE buffer in a calculated amount to achieve concentration 1µg/µl as stock. A working solution of 10pm/µl diluted from stock were used for all further PCR experiments. Primers were optimized for reaction conditions of annealing temperature, Mg concentration, amount of buffer and dNTPs. These optimum conditions were in further experimentation. The following primers was used:

Gene	Primer	GC content (%)	Product Size
TNF α -F	5' CGAGTGACAAGCCTGTAGC 3'	45	45
TNF α -R	5' GGTGTGGGTGAGGAGCACAT 3'	50	3

Table 1: Primer used for PCR

RNA was extracted from blood samples within 6 hours of sample collection. Samples was stored in trizol if extraction is delayed. Samples of extracted RNA were kept at -80°C. RNA quality and quantity were assessed using nanodrop technology. Pcr reaction was followed by gel electrophoresis. The statistical programme SPSS was used for all calculations(version 20.0).

RESULTS

For this research, 80 patients in all were enrolled, and three groups were assigned to them. Group I 40 healthy patients. Group-II 40 patients of T2DM patients without diabetic nephropathy.

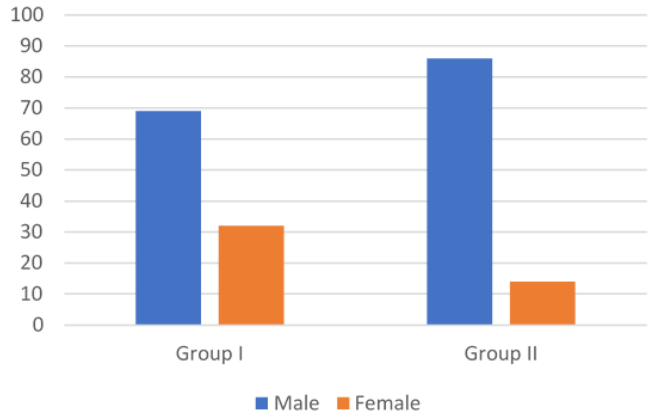

Figure 1: Comparing between Control group and Chronic T2DM

The table 2 presents two groups of 40 individuals each, labeled as Group I and Group II, and provides information about their ages, ALT, and AST levels.

Variables	Group I	Group II
	N= 40	N= 40
Age	47.49 ±2.72	54.99 ± 3.99
ALT	22.71 ± 5.22	66.99 ± 38.47
AST	32.24 ± 7.54	110.09 ± 72.71

Table 2: ALT and AST level in both groups

The figure 2 lists showed subjects along with their corresponding concentration in ng/µl and the amount used for cDNA at 1.5µg.

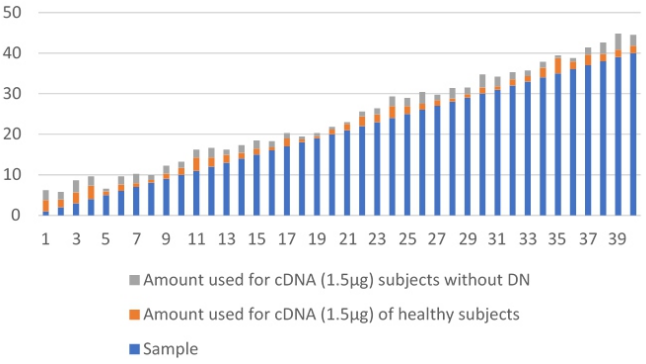

Figure 2: Amount of cDNA used in 2 groups

The impact of T2DM without nephropathy on expression of TNF-a gene was observed by RT-PCR by using gene specific primers and the dye which is SYBR Green mix of the ferments. For internal control GAPDH gene was applied. Each PCR assay of real time was performed in triplicate. Increased expression was observed in T2DM patients with and without nephropathy group as compared to normal group. Almost 4.4 fold induced expression of TNF- a was observed in T2DM patients without nephropathy.

DISCUSSION

TNF-mRNA expression was shown to be low in GMCs that had not yet been triggered by lipopolysaccharide (LPS), according to a research by Affres et al., using the Northern blot technique [13]. In GMC, TNF expression may be induced

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by endotoxin alone as well as by complement fragment, immune complex, platelet-derived growth factor, epidermal growth factor, platelet activating factor, and interleukin [14]. The processes of chronic muscle degeneration and regeneration, which are typical of primary muscle diseases, cause a rise in TNF levels. We aimed to clarify the influence of TNF and IGF1 on gene expression during the early stages of skeletal muscle cell differentiation since pathologically elevated TNF- and IGF1 levels may have either a favourable or detrimental effect on the efficiency of muscle cell differentiation. Many elements, including hormonal imbalances, pathogenic agents, and cytokine gene polymorphisms, control the development and release of cytokines. The genotypes and alleles of the IL-4 gene's -590 region were shown to significantly vary between type 2 diabetes patients with nephropathy and healthy controls [15, 16]. Therefore, based on the current and previous studies, it can be concluded that the expression are associated with nephropathic complications rather than type 2 DM in our studied population by Meloni et al.,[17]. Data showed that there was no relationship between these polymorphisms and type 2 diabetic patients without nephropathy. Many disorders, including glomerulonephritis brought on by immunological complexes, renal lupus, post-aminoglycoside nephritis, rheumatoid arthritis, and septic shock, are mostly caused by TNF. Individual TNF circulating levels differ. Moreover, the rise in circulating TNF levels after the application of an adequate stimulus is substantially larger in some individuals than in others. Activation of the pro-inflammatory cytokine system is elevated in chronic renal failure. Cytokine levels, such as TNF, are significantly elevated in the blood both before and after beginning dialysotherapy. The most significant factor contributing to elevated proinflammatory cytokine levels in dialyzed individuals is likely end-stage renal disease itself [18–20]. It is probably caused by decreased renal clearance and increased cytokine production. Also, it has been shown that TNF- levels are related to the pathological alterations in mesangial proliferative glomerulonephritis (MsPGN), suggesting that TNF- may be a key role in the proliferation, sclerosis, and disease development of GMC. The work by Ozen et al.[16] concentrating on the relationship of TNF- and DN is relevant in light of the significance of TNF- for GMC.

CONCLUSION

In conclusion the implications for the development of new therapies and biomarkers for DN, and for a better understanding of the complex pathophysiology of T2DM.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] International Diabetes Federation. IDF diabetes atlas. International Diabetes Federation (9th edition). 2019. [Last cited: 10th Feb 2023]. Available at: https://diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf.

[2] American Diabetes Association. Standards of medical care in diabetes-2021. Diabetes Care. 2020 Jan; 44(Suppl 1): S1-S2. doi: 10.2337/dc21-Sint.

[3] Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, *et al.* Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care. 2014 Oct; 37(10): 2864-83. doi: 10.2337/dc14-1296.

[4] Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, *et al.* Pathologic classification of diabetic nephropathy. Journal of American Society of Nephrology. 2010 Apr; 21(4): 556-63. doi: 10.1681/ASN.2010010010.

[5] Brosius FC, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, *et al.* Mouse models of diabetic nephropathy. Journal of American Society of Nephrology. 2009 Dec; 20(12): 2503-12. doi: 10.1681/ASN.2009070721.

[6] Donath MY and Shoelson SE. Type 2 diabetes as an inflammatory disease. Nature Reviews Immunology. 2011 Feb; 11(2): 98-107. doi: 10.1038/nri2925.

[7] Bradley JR. TNF-mediated inflammatory disease. Journal of Pathology. 2007 Dec; 214-149-60.

[8] Baud V and Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends in Cell Biology. 2001 Sep; 11(9): 372-7. doi: 10.1016/s0962-8924(01)02064-5.

[9] Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nature Reviews Nephrology. 2011 Jun; 7(6): 327-40. doi: 10.1038/nrneph.2011.51.

[10] Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. Journal of American Society of Nephrology. 2008 Mar; 19(3): 433-42. doi: 10.1681/ASN.2007091048.

[11] Wolf G, Chen S, Han DC, Ziyadeh FN. Leptin and renal disease. American Journal of Kidney Disease. 2002 Jan; 39(1): 1-11. doi: 10.1053/ajkd.2002.29865.

[12] Wada J and Makino H. Inflammation and the pathogenesis of diabetic nephropathy. Clinical Science (London). 2013 Feb; 124(3): 139-52. doi:

DOI: https://doi.org/10.54393/fbt.v2i01.16

10.1042/CS20120198.

[13] Affres H, Perez J, Hagege J, Fouqueray B, Kornprobst M, Ardaillou R, *et al*. Desferrioxamine regulates tumor necrosis factor release in mesangial cells. Kidney International. 1991 May; 39(5): 822-30. doi: 10.1038/ki.1991.103

[14] Kazemi Arababadi M, Reza Mirzaei M, Ali Sajadi SM, Hassanshahi G, Ahmadabadi BN, *et al*. Interleukin(IL)-10 gene polymorphisms are associated with type 2 diabetes with and without nephropathy: a study of patients from the southeast region of Iran. Inflammation. 2012 Jun; 35: 797-802. doi: 10.1007/ s10753-011-9376-7

[15] Golshani H, Haghani K, Dousti M, Bakhtiyari S. Association of TNF-α 308 G/A polymorphism with type 2 diabetes: a case–control study in the iranian kurdish ethnic group. Osong Public Health and Research Perspectives. 2015 Apr; 6(2): 94-9. doi: 10.1016/j.phrp.2015.01.003

[16] Ozen S, Saatci O, Tinaztepe K, Bakkaloglu A, Barut A. Urinary tumor necrosis factor levels in primary glomerulopathies. Nephron. 1994; 66(3): 291-4. doi: 10.1159/000187825

[17] Meloni C, Tatangelo P, Cipriani S, Rossi V, Suraci C, Tozzo C, *et al*. Adequate protein dietary restriction in diabetic and nondiabetic patients with chronic renal failure. Journal of Renal Nutrition. 2004 Oct; 14(4): 208-13. doi: 10.1016/S1051-2276(04)00135-9

[18] Kawanami D, Matoba K, Takeda Y, Nagai Y, Akamine T, Yokota T, *et al*. SGLT2 inhibitors as a therapeutic option for diabetic nephropathy. International Journal of Molecular Sciences. 2017 May; 18(5): 1083. doi: 10.3390/ijms18051083

[19] Kato M and Natarajan R. Diabetic nephropathy—emerging epigenetic mechanisms. Nature Reviews Nephrology. 2014 Sep; 10(9): 517-30. doi: 10.1038/nrneph.2014.116

[20] Gallo E, Cabaleiro T, Román M, Abad-Santos F, Daudén E. Study of genetic polymorphisms in the tumor necrosis factor α promoter region in Spanish patients with psoriasis. Actas Dermo-Sifiliográficas (English Edition). 2012 May; 103(4): 301-7. doi: 10.1016/j.adengl.2012.05.009