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Original Article

Assessment of TNF-α Gene Expression in Type 2 Diabetes Patients with Nephropathy

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ABSTRACT

Type 2 diabetes mellitus (T2DM) and its complications, including nephropathy, are a significant public health concern worldwide. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that plays a crucial role in the development and progression of chronic inflammatory diseases, including T2DM and diabetic nephropathy. **Objective:** To evaluate of TNF-α gene expression in patients with T2DM and nephropathy compared to healthy individuals. **Methods:** The cross-sectional study conducted on 120 individuals divided into three groups: healthy individuals, type 2 diabetes mellitus (T2DM) patients without diabetic nephropathy, and T2DM patients with nephropathy. RNA was extracted and TNF-α gene expression was evaluated using PCR and statistical analysis was done using SPSS software. **Results:** The results showed almost 4.2-fold induced expression of TNF-α in T2DM patients without nephropathy compared to the normal group. **Conclusion:** The study reports that in diabetic nephropathy patients, Gene expression of gene TNF-*α* shows increases in cases when compared with healthy subjects.

INTRODUCTION

Type 2 diabetes (T2D) is the most well-known type of diabetes and an increasingly pervasive metabolic infection. It is related with microvascular and macrovascular complications and is viewed as one of the significant reasons for dismalness and mortality[1-3]. Diabetes can be ordered into four clinical classifications. Type 1 diabetes mellitus, it is safe intervened diabetes which involves 5-10% instances of diabetes. It is named as insulin subordinate diabetes mellitus (IDDM) or adolescent diabetes results from cell interceded resistance [4, 5]. Type 2 diabetes mellitus, it is additionally called grown-up beginning diabetes or non-insulin subordinate diabetes (NIDDM). It involves 90-95% instances of DM. Patients of NIDDM have insulin obstruction because of which there is a family member (when contrasted with supreme) insufficiency of insulin. Gestational diabetes mellitus, it is a brief condition that happens during pregnancy. It influences around of 2-4% everything being equal and includes an expanded danger of creating diabetes for both mother and youngster [6, 7]. A few realities validate the significance of heritability in T2DM: the more noteworthy concordance between monozygotic twins than among dizygotic twins and a wide variety in the pervasiveness of T2DM in epidemiological investigations with various ethnic gatherings just as positive outcomes in various other hereditary examinations [8, 9]. In such manner, it ought to be noticed that even as in excess of 30 qualities related with T2DM have been distinguished, the commitment of every individual quality in the ailment weakness is little. Furthermore, a large portion of these qualities distinguished are identified with brokenness of pancreatic β cells [10-12]. TNF alpha is so important in DM and

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nephropathy so in this study we will analyze gene expression of TNF alpha on PBMC in Diabetes with and without nephropathy as compared to healthy.

METHODS

It was a case-control cross-sectional research. The study was conducted at the Immunology and Resource lab of the University of Health Sciences in Lahore. The sample size for each group is computed to be 40. This research examined a total of 80 participants, who were separated into two groups of 40 persons each. Group I has forty healthy patients. Group II consists of 40 T2DM patients without of diabetic nephropathy. DN was diagnosed based on the presence of microalbuminuria, which was defined as 30 to 300 mg albumin/24 hours or an albumin to creatinine ratio (ACR) of 30 to 300 mg/g, or macroalbuminuria, which was defined as >300 mg albumin/24 hours or an ACR >300mg/g (American Diabetes Association., 2014). Diagnosis of diabetes was made when fasting blood glucose was 126 mg/dl or HbA1c was 6.5%. (ADA., 2014). 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	453
TNF α -R	5' GGTGTGGGTGAGGAGCACAT 3'	50	

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. 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

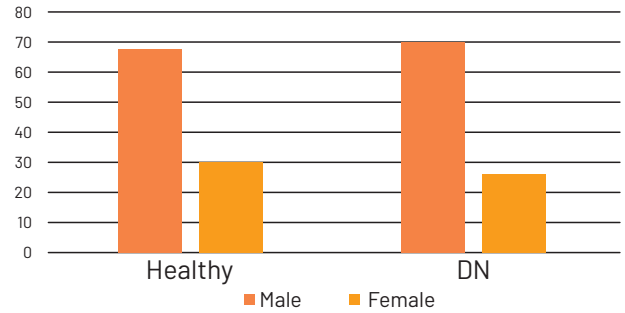

Figure 1: Distribution of male female in both group

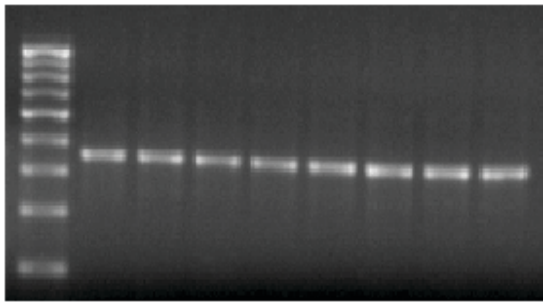

Figure 2: GAPDH PCR Results of cdna Primer optimization (TNF-a)

Correlation between AST, ALT and expression levels of genes TNF-a was carried out through Pearson correlation test. There was a significant negative correlation between levels of ALT and AST and expression levels of TNF-a. Correlation was also computed between two genes and it was found that there is no correlation between in TNF-a cases as well as in controls (Table 2).

	TNF-a	ALT	AST
TNF-*a*	1	-	-
ALT	0.1693	1	-
AST	0.0786	0.6592	1

Table 2: Correlations between AST, ALT, TNF-a

DISCUSSION

TNF- is a proinflammatory cytokine that plays a key role in the development and progression of chronic inflammatory illnesses, such as type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN) [13-15]. T2DM is a metabolic illness defined by elevated blood glucose levels owing to insulin resistance, while DN is a long-term consequence of diabetes that compromises the function of the kidneys. The study of TNF- gene expression in T2DM and DN patients as compared to healthy individuals might give useful insights into the molecular processes underlying the pathophysiology of these illnesses and prospective treatment targets. For quantitative real-time polymerase chain reaction (qRT-PCR) analysis, researchers take blood or tissue samples from participants and extract RNA, which is subsequently reverse-transcribed into complementary DNA (cDNA). qRT-PCR measures the quantity of messenger RNA (mRNA), which corresponds to the level of gene expression. qRT-PCR has been utilised in several studies to analyse TNF- gene expression in T2DM and DN patients. Reidy *et al.*, did a research comparing the gene expression of TNF- in peripheral blood mononuclear cells (PBMCs) between T2DM patients with and without DN and healthy controls [16, 17]. TNF- *a* gene expression was considerably greater in T2DM patients with DN compared to those without DN and healthy controls, as shown by the findings. Kalantarinia *et al.*, investigated the expression of the TNF- gene in renal biopsies from patients with DN and

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healthy controls [18, 19]. TNF- gene expression was considerably greater in renal biopsies from patients with DN compared to healthy controls, according to the research. TNF- *a* gene expression is elevated in T2DM patients with DN relative to those without DN and healthy controls, suggesting a possible role for TNF- in the formation and progression of DN. It is essential to highlight, however, that these studies have limitations, such as limited sample sizes and the possibility of confounding variables, which may alter the interpretation of the findings [20]. Therefore, comparing the TNF- *a* gene expression of patients with T2DM and DN to that of healthy individuals might provide light on the molecular processes underlying the development of these disorders. To validate these results and further understand the function of TNF- *a* in T2DM and DN, however, more investigations with bigger sample numbers and more stringent study designs are required.

CONCLUSIONS

The study reports that in diabetic nephropathy patients, Gene expression of gene TNF-a shows increases in cases when compared with healthy subjects.

Conflicts of Interest

The authors declare no conflict of interest.

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