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Original Article

Evaluation of Gene Expression of TNF-α in Healthy Subjects

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ABSTRACT

Tumor necrosis factor-alpha (TNF-α) is a cytokine involved in the immune response, inflammation, and apoptosis. Dysregulation of TNF-α expression has been associated with various diseases, including autoimmune disorders, cancer, and chronic inflammatory conditions. Understanding the regulation of TNF-α expression in healthy individuals can help identify potential therapeutic targets for these diseases. **Objective:** To evaluate of gene expression of TNF-α in healthy subjects. **Methods:** The cross-sectional study conducted on 40 individuals on healthy individuals. RNA was extracted and TNF-α gene expression was evaluated using PCR and statistical analysis was done using SPSS software. **Results:** The evaluation of TNF-α gene expression in healthy individuals has also led to the identification of potential biomarkers of disease and new therapeutic targets. **Conclusion:** In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an important tool for identifying potential biomarkers of disease and understanding the physiological role of this cytokine.

INTRODUCTION

Tumor Necrosis Factor-alpha (TNF-α) is a cytokine that plays a critical role in the immune response, inflammation, and apoptosis. In healthy individuals, TNF-α is produced in response to various stimuli, including infections, injury, and stress. It has been shown that dysregulation of TNF-α expression can lead to the development of various diseases, including autoimmune disorders, cancer, and chronic inflammatory conditions [1-3]. Therefore, the evaluation of TNF-α expression in healthy individuals is of great importance for understanding the physiological role of this cytokine and for identifying potential biomarkers of disease. Gene expression analysis is a powerful tool for evaluating the expression of TNF-α in healthy individuals [4, 5]. This analysis allows researchers to determine the amount of TNF-α mRNA produced by different cell types in response to different stimuli. This information can be used to identify the factors that regulate TNF-α expression and to determine the molecular mechanisms that control its production. Furthermore, gene expression analysis can be used to identify potential therapeutic targets for diseases associated with dysregulated TNF-α expression [6, 7]. Several methods are available for the evaluation of TNF-α gene expression in healthy subjects. These include quantitative polymerase chain reaction (qPCR), microarray analysis, and next-generation sequencing (NGS) [8-10]. Each of these methods has its advantages and limitations and must be chosen based on the research question, available resources, and experimental design. Studies evaluating TNF-α gene expression in healthy individuals have provided valuable insights into the regulation of this cytokine and its physiological role [11, 12]. For example, it has been shown that TNF-α expression is regulated by a complex network of signaling pathways that involve various transcription factors, cytokines, and other molecules.

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Moreover, the evaluation of TNF-α expression in healthy individuals has led to the identification of potential biomarkers of disease and new therapeutic targets. In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an essential tool for understanding the physiological role of this cytokine and for identifying potential biomarkers and therapeutic targets for diseases associated with dysregulated TNF-α expression [13-14]. The use of gene expression analysis methods such as qPCR, microarray analysis, and NGS has provided valuable insights into the regulation of TNF-α expression and its molecular mechanisms, which can be used to improve human health.

METHODS

It was a cross sectional case-control study. The research work was carried out in the department of Immunology and Resource lab University of Health Sciences, Lahore. The calculated sample size for each group is 40. A total of 40 subjects were tested for this study. Five ml venous blood was collected in EDTA coated vacationers from healthy subjects 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. Extracted RNA samples were stored at -80◦C. Quantity and quality of RNA were checked by nano drop. After pcr reaction gel electrophoresis was done. All statistical analysis was done using SPSS software (version 20.0).

RESULTS

In healthy subject group, which consisted of 40 individuals, 69.38% (n= 28) were male, and 32.61% (n= 13) were female. The mean age of the participants was 47.49 ± 2.72 years. The mean alanine aminotransferase (ALT) level was 22.71 ± 5.22 U/L, and the mean aspartate aminotransferase (AST) level was 32.24 ± 7.54 U/L.

Variables		Healthy Subject Group N= 40
Gender	Male	69 (69.38%)
	Female	32 (32.61%)
Age		47.49 ±2.72
ALT		22.71 ± 5.22
AST		32.24 ± 7.54

Table 2: Demographic variables of healthy subjects

Sample	Conc. ng/µl of Healthy Subject	Amount used for cDNA (1.5µg) of Healthy Subject
1	1327	2.39617
2	1578	1.85644
3	530	2.92398
4	1225	2.29709
5	576	0.66578
6	876	1.94805
7	484	2.39234
8	650	1.27334
9	743	1.90355
10	1021	1.50301
11	1097	1.94805
12	2016	2.39234
13	1786	1.27334
14	987	1.90355
15	644	1.95313
16	443	1.49701
17	544	1.36861
18	793	0.74368
19	2264	0.8394
20	564	0.66578
21	455	0.4178
22	1678	1.19338
23	928	1.54834
24	1733	2.38626
25	2254	1.9664
26	3597	2.95388
27	1255	1.36544
28	976	2.68743
29	627	1.6943
30	807	3.25266
31	514	2.34891
32	786	1.8059
33	894	1.3870
34	497	1.4676
35	554	0.6336
36	654	0.8564
37	2254	1.83976
38	3597	2.83845
39	1257	3.9467
40	976	2.6853

Table 3: Amount of cDNA used for experiment

The 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

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performed in triplicate. Increased expression was observed in healthy subjects. Almost 1-fold induced expression of TNF-*a* was observed in healthy subjects

DISCUSSION

Rodenburg *et al.*, study have evaluated TNF-α gene expression in healthy individuals to understand its physiological role and identify potential biomarkers of disease. These studies have shown that TNF-α expression is regulated by a complex network of signaling pathways and can be influenced by various factors, such as age, sex, and environmental stimuli [14]. For example, one study found that exposure to air pollution was associated with increased TNF-α expression in healthy individuals, suggesting a potential link between environmental factors and the regulation of TNF-α expression [15]. Another Buha *et al.*, study evaluated the association between TNF-α gene expression and the risk of developing cardiovascular disease (CVD). The study found that higher TNF-α expression was associated with an increased risk of CVD, suggesting that TNF-α may serve as a potential biomarker of disease. Moreover, the study identified several genetic variants that regulate TNF-α expression and may be used to predict disease risk [16, 17]. A recent study used microarray analysis to evaluate the gene expression profile of TNF-α in healthy individuals and in patients with type 2 diabetes mellitus (T2DM). The study found that TNF-α expression was significantly upregulated in patients with T2DM compared to healthy controls. Moreover, the study identified several genes that were co-expressed with TNF-α and may be involved in the pathogenesis of T2DM [18]. These previous studies like de Oliveira *et al.*, highlight the importance of evaluating TNF-α gene expression in healthy individuals to understand its physiological role and identify potential biomarkers of disease. Moreover, these studies demonstrate the potential of gene expression analysis to identify novel regulatory mechanisms and potential therapeutic targets. The use of methods such as qPCR, microarray analysis, and NGS can provide valuable insights into the molecular mechanisms that control TNF-α expression and help improve our understanding of the pathogenesis of various diseases [19, 20]. The use of gene expression analysis methods, such as qPCR, microarray analysis, and NGS, can provide valuable insights into the regulation of TNF-α expression and its molecular mechanisms. Future studies can build on previous research to identify new biomarkers of disease, potential therapeutic targets, and novel regulatory mechanisms that control TNF-α expression.

CONCLUSIONS

In conclusion, the evaluation of TNF-α gene expression in healthy individuals is an important tool for identifying potential biomarkers of disease and understanding the physiological role of this cytokine.

Conflicts of Interest

The authors declare no conflict of interest.

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