ORIGINAL RESEARCH

A Comparative Study Of Tnf-Alpha And IL-6 As Potential Biomarkers For Patients With Diabetic Neuropathy

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ABSTRACT

Background &Method: The aim of the study is to compare TNF-alpha and IL-6 as potential biomarkers for patients with diabetic neuropathy. Five ml blood was collected by phlebotomists by venepuncture from patients and healthy controls at the common collection centre. Blood was immediately transferred into 3ml green top heparin vial and 2ml blood into Purple top EDTA vial.Glycatedhemoglobin (HbA1c) levels and Biochemistry were determined for all patients and healthy controls at the clinical Laboratory auto-analyzer. CRP and Insulin assay were done on a fully automatic immunoassay analyser. **Result:** The chi-square statistic is 5.87. The *p*-value is .043131. The result is significant at $p < .05$.The chi-square statistic is 311.8023. The *p*-value is < 0.00001. The result is significant at $p < .05$.The chi-square statistic is 0.7364. The *p*-value is .390818. The result is *not* significant at $p < .05$, for Anthropometric analysis of T2DM cases and Control, Inflammatory mediators in the study group& Immunoassay analysis of study group respectively. **Conclusion:** Our data confirms that TNF-α, IL-6, inflammatory mediator i.e., cytokines, plays a positive role in the pathogenesis of T2DM and can act as early prediction biomarkers which can prevent T2DM in this population. Further studies on the wider range of these inflammatory mediators in association with other biochemical, immunoassay and hematological parameters are needed to establish role of inflammatory markers as early prediction biomarkers which can prevent T2DM in this population. **Keywords:** TNF-alpha, IL-6, biomarkers, diabetic & neuropathy.

INTRODUCTION

TNF-alpha is an inflammatory cytokine, strongly correlated with insulin resistance and chronic inflammation [1].Recently, the molecular mechanisms of TNF-alpha function have been intensively investigated. Many studies demonstrated increased circulating levels of TNF-alpha both in animals [2] and humans, as well as in the retina of diabetic rat. Moreover, TNF-alpha also has a role in the development of insulin resistance; in fact, it affects insulin sensitivity by changing the phosphorylation of insulin receptor substrate-1 and interferes with the insulin signalling cascade, thereby leading to insulin resistance [3].

Diabetes is a growing health problem globally, and type 2 diabetes (T2D) is the most common form of diabetes. A common micro-vascular chronic complication of T2D is diabetic polyneuropathy (DPN) [4].The pathogenesis of DPN is complex and multifactorial and includes an increase in levels of several cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6). Interleukin 6 is a pleiotropic cytokine and a proinflammatory marker. It is essential in the homeostasis of the peripheral nerve [5].Elevated levels of IL-6 induce neuro inflammation leading to the development of pain in DPN. Specific inflammatory mediators are significantly higher in painful DPN than in painless DPN. Inflammatory markers are also higher in T2D patients with peripheral neuropathy than those without peripheral neuropathy.

According to Baka et al., a proinflammatory state might be the common denominator of pain and

peripheralneuropathy in diabetes patients, but the inflammatory profiles seem to differ [6].

Type II diabetes mellitus (T2DM) is a non communicable, chronic disorder and progresses slowly because of multifactorial etiology. T2DM is a leading cause of premature deaths worldwide, and its exception alupsurge poses a severe threat and imposes a huge economicburden worldwide (825 billion dollars per year) [7].According to a recent estimation of the World Health Organization (WHO), 422 million people globally are affectedfrom diabetes with a prevalence rate of 8.5% and 46.3% still remains undiagnosed and the number is projectedto rise to 552 million in 2030. Furthermore, the maximum percentage lives in developing countries and comprises of 40–60 age groups. In 2017 it was reported that India alone has 72 million people affected withT2DM and is projected to rise to 101.2 million in 2030 (2,3). Most of the Indian populations are unaware of that disease. The risk factors of T2DM are suggestively increased with changing lifestyle, blood pressure, central obesity, inadequate physical activity and unhealthy diet [8].

MATERIAL & METHOD

Study Designed: Comparative Study

A total of 200 subjects were included in the study. The T2DM cases and healthy controls were enrolled for the study at Tertiary Care Centre. Among 200 subjects, 90 T2DM patients were finally enrolled in the study. They were diagnosed as per standard of the American Diabetes Association (ADA) criteria and 110 healthy individuals (age-sex matched) were taken as sample controls.

Five ml blood was collected by phlebotomists by venepuncture from patients and healthy controls at the common collection centre. Blood was immediately transferred into 3ml green top heparin vial and 2ml blood into Purple top EDTA vial. Heparinized 3ml blood was centrifuged at 4000 RPM for 2 minutes and plasma was aliquoted into Eppendorf tubes for ELISA and other biochemical analysis stored at -20oc till further analysis.

Glycated haemoglobin (HbA1c) levels and Biochemistry were determined for all patients and healthy controls at the clinical Laboratory auto-analyzer. CRP and Insulin assay were done on a fully automatic immunoassay analyser.

Inclusion criteria: Patients of confirmed diagnosis of T2DM over two years.

Exclusion criteria: Pregnant women, patients suffering from thyroiditis, rheumatoid arthritis, inflammatory bowel syndrome, skin diseases, Weight (kg) was measured by a digital weighing machine.

RESULTS

Table 1: Anthropometric analysis of T2DM cases and Control

Parameters	T2DM Cases (n= 90)	Controls (n=110)	P Value
Age (Years) 48.9±8.4 44.9±9.8	49.8±8.4	46.9±9.8	.043131
Sex (M/F)	46/41	59/51	
BMI (kg/m2)	43.2±7.1	21.2±2.2	
WC(Inches)	44.7±4.6	28.2±6.5	

Data are presented as means ± SD. The significance of the difference is based on the one-way T-test. "BMI: Body mass index; WC: Waist Circumference"

The chi-square statistic is 5.87. The *p*-value is .043131. The result is significant at $p < .05$.

Table 2: Inflammatory mediators in the study group

Parameters	T2DM Cases (n= 90)	Controls (n=110)	P Value
TLC (thousands)	8359±1843	7262±1404	< 0.00001
IL-6 (pg/ml)	18.2±7.2	3.0±2.4	
TNF-α (pg/ml)	33.5±8.8	11.2±1.4	
CRP(mg/dl)	4.1±0.9	1.7±0.9	

Data are presented as means ± SD. The Significance of the difference is based on the one-way T-test. p value < 0.05 are statistically significant. TNF-α: Tumor necrosis factor-Alpha; IL-6: Interleukin-6; CRP: C - reactive protein

The chi-square statistic is 311.8023. The *p*-value is < 0.00001. The result is significant at $p < .05$.

Table 3: Immunoassay analysis of study group

Parameters	T2DM Cases (n= 90)	Controls (n=110)	P Value
Insulin (µu/ml)	33.6±9.1	7.6±7.5	
HOMA-IR	12.8±4.7	1.8±0.7	

Data are presented as means ± SD. The significance of the difference is based on the one-way T-test. p value <0.05 are statistically significant

The chi-square statistic is 0.7364. The *p*-value is .390818. The result is *not* significant at $p < .05$.

DISCUSSION

Diabetic peripheral neuropathy (DPN) is the most common chronic neurological complication of diabetes [9]. Small and large peripheral nerve fibers can be involved in DPN. Large nerve fiber damage causes paresthesia, sensory loss and muscle weakness, and small nerve fiber damage is associated with pain, anaesthesia, foot ulcer and autonomic symptoms. However, the exact pathogenic mechanisms and diagnostic criteria for DPN are currently not firmly established [10]. The present DPN prevention,

diagnosis and treatment strategies are incomplete and unsuccessful due to the various forms of pathogenesis of systemic and cellular disturbance in glucose and lipid metabolism [11]. These abnormalities lead to the activation of complex biochemical pathways, including increased oxidative–nitrosative stress, activation of the polyol and protein kinase C pathways, activation of polyadenosine diphosphate ribosylation, and activation of genes involved in neuronal damage, cyclooxygenase-2 activation, endothelial dysfunction, altered Na+/K+-adenosine triphosphatase pump function, impaired C-peptide-related signalling pathways, endoplasmic reticulum stress, and low-grade inflammation [12]. From these pathophysiological factors responsible for DPN, oxidative–nitrosative stress and inflammation are the most extensively studied pathways [13].

Worldwide, people are suffering from T2DM and it is projected to increase from the present 415 million peopleto 642 million by 2040 [14]. In all developing countries, it was seen that the number of T2DM patients is increasing and 75% of people with T2DM are living in these developing countries [15]. In this study, we observed that socio-demographic factors like education, lifestyle and smoking have significant associations with T2DM except for residence (urban and rural areas of the same geographical area) which had no substantial influence on the levels of inflammatory mediators of study like, TNF-α, CRP, IL-6and WBC. After a thorough literature review on the dynamics of T2DM and inflammatory mediators [16]

CONCLUSION

Our data confirms that TNF-α, IL-6, inflammatory mediator i.e., cytokines, plays a positive role in the pathogenesis of T2DM and can act as early prediction biomarkers which can prevent T2DM in this population. Further studies on the wider range of these inflammatory mediators in association with other biochemical, immunoassay and haematological parameters are needed to establish role of inflammatory markers as early prediction biomarkers which can prevent T2DM in this population.

Conflicts of Interest: None
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REFERENCES

1. Moore K, Malefyt R, Coffman R, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19 (1): 683-765.
2. Beyaert R, Fiers W. Tumor necrosis factor and lymphotoxin. In: Mire-Sluis A, Thorpe R, (eds.). Cytokines. 2nd ed. London: Academic Press; 1999. p. 335-45.
3. Shan K, Kurrelmeyer K, Seta Y, Wang F, Dibbs Z, Deswal A, et al. The role of cytokines in disease progression in heart failure.Curr Opin Cardiol. 1997; 12 (3): 218-23.
4. Anker S, Steinborn W, Strassburg S. Cardiac cachexia. Ann Med. 2004; 36 (7): 518-29.
5. Sharma R, Coats AJS, Anker SD. The role of inflammatory mediators in chronic heart failure: cytokines, nitric oxide, and endothelin-1. Int J Cardiol. 2000; 72 (2): 175-86.
6. Kang Y-M, Ma Y, Elks C, Zheng JP, Yang ZM, Francis J. Cross-talk between cytokines and renin-angiotensin in hypothalamic paraventricular nucleus in heart failure: role of nuclear factor-{kappa}B. Cardiovasc Res. 2008; 79 (4): 671-8.
7. Zhang Z-H, Wei S-G, Francis J, Felder RB. Cardiovascular and renal sympathetic activation by blood-borne TNF-alpha in rat: the role of central prostaglandins. Am J Physiol RegulIntegr Comp Physiol. 2003; 284 (4): R916-27.
8. Francis J, Wei S-G, Weiss RM, Felder RB. Brain angiotensin-converting enzyme activity and autonomic regulation in heart failure. Am J Physiol Heart Circ Physiol. 2004; 287 (5): H2138-46.
9. Adamopoulos S, Parissis JT, Kremastinos DT. A glossary of circulating cytokines in chronic heart failure.Eur J Heart Fail. 2001; 3 (5): 517-26.
10. Ganesh Yerra V, Negi G, Sharma SS,et al. Potential therapeutic effects of the simultaneous targeting of the Nrf2and NF-jB pathways in diabetic neuropathy. Redox Biol 2013; 1: 394–397.
11. Herder C, Kannen berg JM, Huth C, et al. Proinflammatory cytokines predict the incidence and progression of distal sensori motor polyneuropathy: KORA F4/FF4 study. Diabetes Care2017; 40: 569–576.
12. G. A. Limb, H. Soomro, S. Janikoun, R. D. Hollifield, and J. Shilling, "Evidence for control of tumour necrosis factor-alpha (TNF-α) activity by TNF receptors in patients with proliferative diabetic retinopathy," *Clinical and Experimental Immunology*, vol. 115, no. 3, pp. 409–414, 1999.
13. Moriwaki Y, Yamamoto T, Shibutani Y, Aoki E, Tsutsumi Z, etal TS. Elevated levels of interleukin-18 and tumor necrosis factor-[alpha] in serum of patients with type 2 diabetes mellitus: relationship with diabetic nephropathy. Metab Clin Exp. 2003; 52:605–8.
14. Mather KJ, Funahashi T, Matsuzawa Y, Edelstein S, Bray GA, Kahn SE, et al. Adiponectin, change in adiponectin, and progression to diabetes in the Diabetes Prevention Program. Diabetes. 2008; 57:980–6.
15. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA.2001; 286:327–34.
16. Kaur K, Sharma AK, Dhingra S, Singra PK. Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. J Mol Cell Cardiol. 2006; 41(6):1023-30.