ORIGINAL RESEARCH PAPER

Medicine

Expression and Association of Tumour Necrosis Factor-alpha and Insulin Resistance in obese subjects with type II diabetes mellitus at tertiary care hospital in Jhansi (UP).

KEY WORDS:TNF-α, Diabetes, obesity, insulin resistance.

Vimal Arya Assistant professor, Department of Community Medicine, MLBMC, Jhansi (UP)

Shubhanshu Gupta Post graduate, Department of Community Medicine, MLBMC, Jhansi (UP)

ABSTRACT

Background- The burden of Diabetes Mellitus (DM) is increasing worldwide and it is estimated to reach indefinite proportions of about 450 million by year 2030.
Objectives - To investigate the changing levels of TNF-α, IR, Hb1Ac, BMI, and blood lipid profiles in diabetic patients with obesity and to analyze the co-relation of TNF-α with insulin resistance and blood lipid profiles.
Material and Methods- It was a hospital based prospective study conducted at outpatient department of MLB medical college. Patients with complication of diabetes like diabetes neuropathy, nephropathy, retinopathy etc. were excluded. Epi-info software was used for statistical analysis.
Results- There is a significant difference in TNF-α, IR, HbA1c, total cholesterol and HDL. TNF-α is significantly associated with IR with group 1.
Conclusion- TNF-α may be involved in the etiology of insulin resistance in type II diabetes mellitus with obesity.

Introduction

Diabetes mellitus (DM) is the most widespread complex metabolic disorder among the world's population currently affecting around 250 million people globally [1]. The burden of Diabetes Mellitus (DM) is increasing worldwide and it is estimated to reach indefinite proportions of about 450 million by year 2030. This complicated metabolic syndrome is due to either insulin insufficiency or impaired action of the insulin hormone or both (American Diabetes Association, 2004) [2]. Insulin resistance and a deficiency in insulin secretion are the major cellular basis of T2DM [3]. The action of insulin is to lower the glucose levels in the blood and to stimulate the uptake of glucose principally in muscle and liver cells, thus involved in promoting glucose oxidation and glycogenesis [4]. TNF-**α** is primarily secreted by macrophages, and also by a broad variety of other cells including adipocytes [5]. Tumour necrosis factor alpha (TNF-α) is an adipocytokine involved in systemic in ammation and stimulates the acute phase reaction [6]. TNFα is now acknowledged as being a pluripotent cytokine, and the mechanisms of many of its biological activities are still not clearly understood. It is known that TNFα can cause apoptosis, septic shock, inflammation and cachexia systemically [5]. Disturbances in TNF-α have been seen in metabolic disorders such as obesity and insulin resistance that shows that changes of TNF-α metabolism may affect the onset of type II DM and CVD [7]. HbA1c is a marker of cumulative glycaemic exposure over the preceding two to three months in diabetic patients. The aim of this study was to investigate the changing levels of TNF-α, IR, Hb1Ac, BMI, and blood lipid profiles in diabetic patients with obesity and to analyze the co-relation of TNF-α with insulin resistance and blood lipid profiles.

Material and Methods

It was a hospital based prospective record retrieval study conducted over a period of 8 months from November 2015 to June 2016. A total of 100 diabetic patients with obesity were selected with the mean age of 54.2±12.4 years from the outpatient department of department of internal medicine, MLB medical college, Jhansi (UP). The subjects were arbitrarily categorized into three groups based on duration of diabetes with obesity. Group 1 included subjects of ≤5 year duration, group 2 included diabetics with obesity of 6-10 year duration and group 3 with > 10 years duration.

Inclusion criteria-1. Diabetic patients of either sex between 40-75 years

2. Subjects those who are currently not on any oral hypoglycemic agents (OHA)

Exclusion criteria-1. Patients with complication of diabetes like diabetes neuropathy, nephropathy, retinopathy etc. were excluded

2. Patient on smoking and alcohol are excluded.
3. Patient with any systemic disease, hypertension or any heart disease based on clinical or laboratory investigation.

Anthropometric measurements: Height was noted using a measuring tape (to the nearest 0.1 cm), with the subjects wearing light clothes and no shoes. Weight was measured to the nearest 0.1 kg using a mechanical weighing machine. BMI, defined as mass in kilograms divided by the square of height in meters, was calculated.

Homeostatic Model Assessment (HOMA) method, which has been validated as a reliable measure of insulin sensitivity in humans [12] was used to estimate insulin resistance (HOMA IR).

Biochemical parameters: After overnight fasting blood samples were obtained by venipuncture. The serum was separated and stored at -20°C. Serum TNF-α was quantified using sandwich ELISA kit from eBioscience. HbA1c and blood lipid profiles were all measured at the same time. Fasting serum glucose concentration was measured using an enzymatic reaction and also used to measure triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL). HbA1c was quantitatively determined by ELISA. All of biochemical tests were done using the reagents from diagnostic machine at MLB hospital. The Ethical Committee members of the hospital were briefed about the rationale of the study, nature of the procedures, and the benefits that could be availed. There being neither any ethical considerations nor conflicts of interest, the ethical committee clearance was obtained. Consent from the patients was also obtained.

Statistical analysis- Epi-info software was used and results or continuous variables are given as mean and SD. Unpaired student t-test is used to check the significant difference between three groups of diabetic patients with obesity. Spearman's correlation was used to estimate the association between the variables.

Results and observations-

Table 1- Physical parameters of the study groups

Parameters	Group 1(n=40)	Group 2(n=38)	Group 3 (n=22)
Age	54.212.4	51.610.2	53.414.6
BMI (kg/m^2)	251.4	25.41.6	27.22.4

Duration of diabetes with obesity	3.21.4	7.22.2	11.48.6

All values are in mean± SD (standard deviation)

Table 1 depicts about the physical parameters of respondent's shows three variables age, BMI, and duration of diabetes with obesity. The mean age of respondents who had duration of diabetes with obesity of ≤ 5 years is 54.2±12.4. BMI is increased according to the duration i.e. patient with duration > 10 years has higher BMI compared to those whose duration is less.

Table 2- Level of changes among different biochemical parameters in three groups

Biochemical parameters	Group 1 (n=40)	Group 2 (n=38)	Group 3 (n=22)	p-value*
TNF-α	1.070.2	1.60.4	1.91.2	**0.03**
IR (insulin resistance)	0.050.65	0.240.49	0.120.56	**0.01**
HbA1c	6.80.2	7.20.8	8.82.2	**0.001**
Total cholesterol	4.41.2	5.82.4	6.42.8	**0.04**
Triglycerides	1.10.8	1.61.2	2.21.6	0.68
LDL	2.40.98	3.20.97	4.81.2	0.76
HDL	1.40.34	0.980.22	0.740.16	**0.03**

- p-value <0.05 is considered statistically significant.

Table 2 depicts level of changes of different biochemical parameters in three groups; there is a significant difference in TNF-α, IR, HbA1c, total cholesterol and HDL. HbA1c of diabetic patient with obesity >10 year is higher which shows glycosylated hemoglobin is time dependent. Lipid profile is done to represent relationship between subjects for higher risk of coronary heart disease (CHD).

Table 3- Association of TNF-α with insulin resistance and lipid profiles parameters

Correlation	Group1 (n=40)	Group2 (n=38)	Group3 (n=22)
TNF-α vs HOMA IR	P= 0.014*	P=0.619	P=0.788
TNF-α vs TG	P= 0.456	P= 0.558	P= 0.012*
TNF-α vs TC	P=1.2	P=0.987	P= 0.04*
TNF-α vs HDL	P=0.02*	P=0.324	P=0.873
TNF-α vs LDL	P=0.889	P=0.761	P=0.04*

*indicates statistically significant values.

Table 3 shows spearman's correlation that shows relationship of TNF-α with IR and lipid profile parameters. TNF-α is significantly associated with IR with group 1, while with triglycerides, total cholesterol and LDL it is significantly associated with group 3 respondents.

Discussion

The OPD of MLBMC, Jhansi shows a good patient output. Since there is dearth in the literature as no other study was conducted before in this region to show the expression of TNF-α and IR with obesity in diabetic patient this study was conducted. Our results are similar to the study conducted by JJ Swaroop et al [8] that shows significant correlation between with HOMA IR with group 2 diabetes. Miyazaki et al [9] have concluded that TNF- increased before the onset of diabetes and further increase was not significantly associated with insulin resistance. Bluher et al [10] reported no significant association of TNF-α in the genesis of early stages of insulin resistance. Demirbas et al [11] showed that in patients with hypertension serum TNF-α concentration increased together with increase in concentrations of insulin, and HOMA IR. No correlations were found between insulin resistance and TNF-α. Few studies revealed that increased adipose expression of TNF-α mRNA in a non-diabetic subjects with obesity dependency on IR, and in patients with normal glucose levels increased IR dependency and in type II diabetes [12]. TNF-α also inhibits synthesis of specific protein associated with adipocytes like adiponectin and increased levels of free fatty acids [13]. In this study it has been found that HbA1c increases as the duration of diabetes with obesity also increases. The elevated HbA1c percentage in diabetic patients is indicative of poor glycemic control that is an indicative of increased risk of coronary heart disease. In our study results of blood lipid profile shows a significant difference in groups as compared to similar study conducted by M H saiem [14]. TNF-α may play a potentially important pathophysiological role in the development of insulin resistance, particularly in males and in people with high BMI with raised HbA1c.

Conclusions

Recorded observations in our study support the hypothesis that TNF-α may be involved in the etiology of insulin resistance in type II diabetes mellitus with obesity. Significant association of TNF-α with blood lipid profile markers also suggest the risk of cardiovascular complications.

Acknowledgement- the authors are very thankful to HOD, department of internal medicine and Head Department of Biochemistry Dr. Prabhakar Singh Bais, support staff of the medical college who provide us uninterrupted support to conduct our research.

Conflict of interest- none declared.

References

1. Srinivasan BT, Jarvis J, Khunti K, & Davies MJ (2008). Recent advances in the management of type 2 diabetes mellitus: a review. Postgraduate Medical Journal 84, 524-531.
2. American Diabetes Association (2004). Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 27, s5-s10.
3. Mahler RJ & Adler ML (1999). Type 2 Diabetes Mellitus: Update on Diagnosis, Pathophysiology, and Treatment. J Clin Endocrinol Metab 84, 1165-1171.
4. Johnson EL, Brosseau JD, Soule M, & Kolberg J (2008). Treatment of Diabetes in Long-Term Care Facilities: A Primary Care Approach. Clinical Diabetes 26, 152-156.
5. Beutler B, Cerami A. The biology of cachectin/TNF- primary mediator of thel host response Ann Rev Immunol 1989; 7 : 625-55.
6. Moller DE. Potential role of TNF alpha in the pathogenesis of insulin resistance and type 2 diabetes. Ternds Endocrinol Metab 2000; 11 : 212-7.
7. Zou C, Shao J. Role of adipocytokines in obesity- associated insulin resistance. J Nutr Biochem 2008; 19 : 277-86.
8. JJ Swaroop, D rajarajeshwari, JN Naidu. Association of TNF- with insulin resistance in type 2 diabetes mellitus. Indian J Med R. 2012 Jan: 135(1): 127-130.
9. Miyazaki y, Pipek R, Mandarino LJ, DeFronzo RA. Tumor necrosis factor alpha and insulin resistance in obese type 2 diabetic patients. Int J Obes Relat Metab Disord 2003; 27 : 88-94.
10. Bluher M, Kratzsch J, Paschke R. Plasma levels of tumor necrosis factor-alpha, angiotensin II, Growth harmone, and IGF- 1 are not elevated in insulin-resistant obese individuals with impaired glucose tolerance. Diabetes Care 2001; 24 : 328-34.
11. Demirbas B, Guler S, Cakir B, Culha C, Aral y. Plasma tumor necrosis factor-alpha levels and insulin resistance in nondiabetic hypertensive subjects. Horm Res 2002; 58 : 283-6.
12. Hotamisligil S, N Shargill, M Spigelman. Adipose expression of TNF- : direct role in obesity linked insulin resistance. Science 1993; 259:87-91.
13. American Diabetes Association, 2006. Type 2 diabetes in children and adolescents. Pediatrics, 105 (3Pt1):671-680.
14. M H Saiem, E H Jiffri. Increased adipose tissue expression of tumour necrosis factor-alpha and insulin resistance in obese subjects with type II diabetes. World journal of medical sciences.2010; 5(2): 30-35.