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EXPRESSION AND ASSOCIATION OF TUMOUR NECROSIS FACTOR-ALPHA AND INSULIN RESISTANCE IN OBESE SUBJECTS WITH TYPE II DIABETES MELLITUS

Biochemistry

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ABSTRACT

BACKGROUND- The burden of Diabetes Mellitus 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 tumor necrosis factor-alpha, insulin resistance, glycosylated hemoglobin , body mass index and blood lipid profiles in diabetic patients with obesity and to analyze the co-relation of Tumor necrosis factor with insulin resistance and blood lipid profiles.

MATERIAL AND METHODS- It was a hospital based study conducted in the department of biochemistry at People's College of Medical Science & Research Centre Bhopal (M.P.). 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 tumor necrosis factor- alpha, insulin resistance, glycosylated hemoglobin , total cholesterol and high density lipoprotein. Tumor necrosis factor-alpha is significantly associated with insulin resistance with group 1.

CONCLUSION- Tumor necrosis factor- alpha may be involved in the etiology of insulin resistance in type II diabetes mellitus with obesity.

KEYWORDS

TNF-α, Diabetes, obesity, insulin resistance.

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 inflammation and stimulates the acute phase reaction [6]. TNF-α 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]. HbA_{1c} 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, $Hb1A_c$, 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-

The study was conducted in the department of biochemistry at People's College of Medical Science & Research Centre Bhopal. For the purpose of study 70 cases were selected as per iclusion and exclusion criteria with the help of physicians during the period January 2017 to August 2017 from people's hospital. The subjects were arbitrarily categorized into three groups based on duration of diabetes with obesity. Group 1 included subjects of $\leq$ 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 vein puncture. The serum was separated and stored at -20°C. Serum TNF-α was quantified using sandwich ELISA kit . Fasting glucose, 2-hr glucose, HbA1c and lipid profile were analyzed by Cobas c-311 fully automated autoanalyzer (Roche diagnostics).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=30)	Group 2(n=28)	Group 3 (n=12)
Age	54.2 + 12.4	51.6+10.2	53.4+14.6
BMI (kg/m^2)	25+1.4	25.4+1.6	27.2+2.4
Duration of diabetes with obesity	3.2 + 1.4	7.2+2.2	11.4+8.6

*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=30)	Group 2 (n=28)	Group 3 (n=12)	p-value*
TNF-α	1.07±0.2	1.6±0.4	1.9±1.2	0.03
IR (insulin resistance)	0.05±0.65	0.24±0.49	0.12±0.56	0.01
HbA1c	6.8±0.2	7.2±0.8	8.8±2.2	0.001
Total cholesterol	4.4±1.2	5.8±2.4	6.4±2.8	0.04
Triglycerides	1.1±0.8	1.6±1.2	2.2±1.6	0.68
LDL	2.4±0.98	3.2±0.97	4.8±1.2	0.76
HDL	1.4±0.34	0.98±0.22	0.74±0.16	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=30)	Group2 (n=28)	Group3 (n=12)
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-

People's College of medical Science & Research Center Bhopal, 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**.** TNF-α is a pro-inflammatory cytokine that exerts numerous effects in adipose tissue including lipid metabolism and insulin signaling whose circulating levels are increased with obesity and decreased with weight loss. An increase in TNF-α promotes the secretion of other pro-inflammatory cytokines IL-6 and TNF-α, and reduces anti-inflammatory cytokines like adiponectin . Evidence suggests that TNF-α induces adipocytes apoptosis and promotes insulin resistance by the inhibition of the insulin receptor substrate 1 signaling pathway . Miyazaki *et al* [9] have concluded that TNF-α 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.

Conflict of interest- none declared.

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