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Research Article

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Exploration of the relationship between adipocytokines, antioxidant vitamins and obese diabetic patients

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ABSTRACT

This study was performed to find out the relationship between the adipocytokines and antioxidant vitamins in the development of type 2 diabetes mellitus (T2DM). 45 subjects divided into 3 groups (15 for each group): normal subjects, diabetic subjects, obese diabetic subjects. Serum glucose, lipid profile, insulin, adipocytokines including (leptin, adiponectin, resistin) and antioxidant vitamins (A&E) were estimated. The mean values of Serum insulin and leptin levels were significantly higher in obese diabetics while significantly lower in diabetics compared to the control group. In contrast, adiponectin concentration decreased significantly in diabetic and obese diabetic groups when compared to the control. No significant difference in serum resistin level between the three groups. There was a significant decrease in vitamin A level in both diabetic and obese diabetic groups as compared to the control group. Whereas, there was none statistically significant decrease in serum level of vitamin E in both II & III groups comparing to controls. These results indicated the importance of taking these adipocytokines in consideration as biomarkers for T2DM and obesity during treatment. We suggest the inclusion of dietary supplementation of the antioxidant vitamins A & E in the management of type 2 diabetes mellitus.

Keywords: Diabetes, Obesity, Adiponectin, Leptin, Resistin, Vitamin A, Vitamin E

INTRODUCTION

Adipose tissue is not just an energy storage organ but it is an active endocrine organ, secreting numerous of bioactive mediators termed as adipocytokines. These adipocytokines include several novel and highly active molecules released plentifully by adipocytes like resistin, leptin, adiponectine or visfatin as well as more classical cytokines secreted possibly by inflammatory cells, like IL-6 (interleukin-6) and TNF-a (Tumor Necrosis Factor Alpha) [1]. Adipocytokines affects many biological activities including hypertension, diabetes, obesity and cardiovascular diseases. These factors may represent a link between obesity, diabetes, inflammation and atherosclerosis [2].

Adiponectin appears to be a second well known adipocytokine released by fat cells but in contrast to leptin it seems to have several beneficial and protective effects. These effects include anti-inflammatory, vasculoprotective and anti-diabetic effects [3]. Level of adiponectin in human blood are decreased in subjects with insulin resistance and type 2 diabetes and is negatively correlated with body mass index in contrast to the markedly increased levels of leptin, resistin or TNF-a in obesity [4]. High adiponectin levels should protect against impairments of glucose metabolism moreover, administration of adiponectin causes glucose-lowering effects and ameliorates insulin resistance [5].

The adipocytokine, leptin is a peptide hormone secreted principally but not exclusively by adipocytes. It plays an important role in the central regulation of food intake and energy expenditure [6]. Although a strong correlation between serum insulin and levels leptin has been demonstrated in human studies [7] its role in type 2 diabetes is not yet clear.

Another adipocyte-derived hormone is resistin, a member of newly discovered cysteine rich secretary protein family. Initial studies in rodents suggest that it may be involved in the development of insulin resistance [8]. However, later studies failed to confirm this hypothesis [9]. Thus, the role of resistin in relation to insulin resistance and obesity is questionable.

Diabetes mellitus is characterized by elevated level of oxidative stress indices, decreased levels of antioxidants defenses and lipid abnormalities due to lipid peroxidation [10]. Antioxidant vitamins and minerals are thought to be effective in increasing the activities of antioxidant defense enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation [11].

Epidemiological evidence suggests that serum vitamin A, C and E, chromium, manganese and zinc, are potent antioxidants play a protective role in the development of chronic diseases including diabetes, cancers, cardiovascular diseases, and inflammatory diseases [12].

When the researchers removed vitamin A from the diets of healthy mice, they found that this led to significant beta cell loss, resulting in reduced insulin production and increased blood glucose levels- key factors involved in the development of type 2diabetes. When they restored vitamin A to the rodents' diet, beta cell production rose, and insulin production increased and blood glucose levels returned to normal. The researchers said that their findings indicate that vitamin A deficiency may be involved in the development of type 2 diabetes [13].

People with diabetes have a higher than usual need for vitamin E, which improves insulin activity and acts as an antioxidant and a blood oxygenator. Research has shown that people with low blood levels of vitamin E are more likely to develop type 2 diabetes. Double blind study also showed that vitamin E improves glucose tolerance in people with type 2 diabetes (NIDDM) [14].

This study was designed to find out the relationship between adipocytokines and antioxidant vitamins (A & E) in the development of type 2 diabetes mellitus**.**

EXPERIMENTAL SECTION

I-Subjects:

The study was carried out on 45 subjects aged (18-40), recruited from obesity and internal medicine clinics of the National Research Centre. Subjects were divided into three groups:

Group I: 15 normal subjects.
Group II: 15 diabetic subjects.
Group III: 15 obese diabetic subjects.

Exclusion criteria were presence of diabetic complications, celiac disease, liver disease, presence of any associated musculoskeletal or metabolic bone diseases, any chronic illness other than diabetes, any medications other than insulin, and delayed puberty. The study was approved by the ethics committee of the National Research Centre and all subjects gave their informed consent prior to entering this study.

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II Methods:

a) Anthropometry:

Anthropometric measurements, including height and weight were done **[**15]. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared (m^2).

b) Biochemical Assays:

Venous blood samples were collected from subjects after 10-12 hours fasting; samples were left to clot, and centrifuged at 4000 r.p.m by cooling centrifuge for 15 minutes to separate the sera. Determination of fasting blood glucose was done within 2 hours of blood collection by the glucose peroxidase method**, [**16] then serum was stored

at -20°C until used for estimation of lipid profile, insulin, adipocytokines including (leptin, adiponectin, resistin) and vitamins (A&E). Serum cholesterol [17], high density lipoprotein (HDL-cholesterol) [18], low density lipoprotein (LDL-cholesterol) [19] and triglycerides [20] were measured by the enzymatic method; the kits were supplied from Biocon Diagnostic (Germany).

Serum concentrations of insulin, leptin, adiponectin and resistin were assayed by an enzyme-linked immunosorbent assay (ELISA). Insulin and leptin were measured according to Judzewitsch et al., [21]; Considine et al., [22] respectively and the kits were from (DRG, USA). Adiponectin was assayed according to Watanabe et al., [23] and the kit was supplied from (Orgenium Laboratories, Finland). Resistin was measured according to Pilz et al., [24] with a kit supplied from (Biovendor, Czech Republic).

Analysis of vitamins A and E by high performance liquid chromatography (HPLC):

Sample extraction:

One hundred µl of serum was mixed with ethanol. The micronutrients were extracted from the aqueous phase in hexane and dried under vacuum. The extract was re-dissolved in ethanol and acetonitrile and filtered to remove any insoluble materials.

HPLC condition for vitamin A

Twenty µl of the filtrate were injected onto a C18 reversed phase column (25cm×10.00 mm, 5 µm particle size) and isocratically eluted with a mobile phase consisting of ethanol/acetonitrile 50:50 with 0.1% trimethylamine , and was delivered at a flow rate of 1 ml/min. UV detection was performed at 325 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas *vs* the corresponding concentrations. The concentrations in samples were obtained from the curve.

HPLC condition for vitamin E

Twenty µl of the filtrate were injected onto a C18 reversed phase column (15cm×10.00 mm, 5 µm particle size) and the thermostat was adjusted to 30ºC with a mobile phase consisting of 100% methanol delivered at a flow rate of 1 ml/min. Fluorescence detector was used and performed at 295 and 330 (excitation and emission). Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas *vs* the corresponding concentrations. The concentrations in samples were obtained from the curve.

Statistical Analysis

All analysis was done using the statistical package for the social science (SPSS) software version 9 on a personal computer. All numeric variables were expressed as a mean ± standard deviation (SD). The independent-sample T test was used to compare means. Pearson's correlation coefficient was obtained and a 'p'value<0.05 was considered as statistically significant. Step wise multiple regressions were applied to illustrate relationship between several independent or predictor variables and a dependent or criterion variable. The Beta (standardized regression coefficients) value is a measure of how strongly each predictor variable influences the criterion variable. P value <0.05 was considered as an entrance criterion, while p >0.05 was considered as removal criterion.

RESULTS AND DISCUSSION

Clinical and metabolic parameters:

With respect to clinical characteristics, the results show that BMI was significantly higher among obese diabetics compared to control and diabetic groups (table1).

As for metabolic parameters it is clear that fasting blood glucose was significantly higher in diabetic and obese diabetic groups compared to control group while there was no significant difference between group II and group III (table 1). For total cholesterol and LDL-cholesterol the difference was significantly higher in diabetic and obese diabetic groups comparing with the control. Also the difference was statistically significant between diabetic and obese diabetic groups. HDL-cholesterol was significantly lower in the diabetic and obese diabetic groups compared to controls, significant decrease in the mean level of HDL-cholesterol was observed in obese diabetic group compared to diabetic group. Significant increase in the mean level of triglycerides was observed only in obese diabetics, while significant increase in the mean level of VLDL-cholesterol in both II & III groups compared to controls (table 1).

Serum insulin and leptin levels were significantly higher in obese diabetics while significantly lower in diabetics compared to the control group. In contrast, adiponectin concentration decreased significantly in diabetic and obese diabetic groups when compared to the control. No significant difference in serum resistin level between the three groups (table 2). In (table 3), there was a significant decrease in vitamin A level in both diabetic and obese diabetic

groups as compared to the control group. Whereas, there was none statistically significant decrease in serum level of vitamin E in both II & III groups comparing to controls.

Table (1): Body mass index, fasting blood glucose and lipid profile in the different studied groups

Groups / Parameters	Group I Control n=15	Group II Diabetics n=15	Group III Obese Diabetics n=15
Body mass index (BMI) Kg/m^2	23.3 ± 1.86	24.1 ± 3.81	35.24 ± 2.26 [ab]
Fasting blood glucose M mol /l	4.09 ± 0.33	7.55 ± 0.57 [a]	8.85 ± 2.48 [a]
Total cholesterol mg/dl	166.47±17.21	193.53 ± 18.41[a]	232.4±15.87 [ab]
Triglycerides mg/dl	101.73 ± 13.32	127.12±39	137.23±24.08[a]
HDL-cholesterol mg/dl	56.86 ± 14.9	40.86 ± 8.7 [a]	32.73±13.36 [ab]
LDL-cholesterol mg/dl	89.26 ± 13.74	127.25 ± 18.39 [a]	172.22±29.25 [ab]
VLDL –cholesterol mg/dl	20.34 ± 0.22	25.42 ± 0.31 [a]	27.44 ± 0.32 [a]

Values are mean ±SD where, a: Significant difference compared to group I (p< 0.05).
b: Significant difference compared to group II (p< 0.05).

Table (2): Insulin, leptin, adiponectin and resistin in the different studied groups

Groups / Parameters	Group I Control n=15	Group II Diabetics n=15	Group III Obese diabetics n=15
Insulin µ IU/ml	10.54 ± 3.45	7.99 ± 4.68 [a]	17.15 ± 8.91 [ab]
Leptin ng/ml	11.51 ± 2.95	6.68 ± 3.53[a]	23.23± 4.98 [ab]
Adiponectin ng/ml	8.53 ± 2.42	5.79 ± 1.80[a]	4.75 ±1.04 [a]
Resistin ng/ml	10.22±1.67	9.27±0.61	11.84 ± 1.95

Values are Mean ±SD where, a: Significant difference compared to group I (p<0.05). b: Significant difference compared to group II (p<0.05).

Table (3): Vitamin A and vitamin E in the different studied groups

Groups / Parameters	Group I Control n=15	Group II Diabetics n=15	Group III Obese diabetics n=15
Vitamin A µg/dl	42.22 ± 12.65	24.79 ± 7.53 [a]	22.23± 4.98 [a]
Vitamin E mg/dl	0.63 ± 0.12	0.53 ± 0.20	0.49 ±0.19

Values are Mean ±SD where,
a: Significant difference compared to group I (p<0.05).

Obesity and obesity related diseases (T2DM and CVD) are major public health problems. Recent studies have shown that fat tissue is not simple energy storage organ but exerts important endocrine and immune functions. These are achieved predominantly through release of adipocytokines like leptin, adiponectin, resistin, TNF_{α} and IL6. All of these molecules may act on immune cells leading to local and generalized inflammation and results in obesity related disorders including insulin resistance, diabetes, hypertension and atherosclerosis. Though these adipocytokines are proposed to link obesity and diabetes their relationship with each other and their interplay are still poorly understood [25].

In this study, we analyzed the impact of obesity and T2DM on adipocytokines (adiponectin, leptin and resistin). The present study confirmed previous findings that obesity and T2DM are associated with low plasma adiponectin concentrations. We indicated that non-obese T2DM patients have lower plasma adiponectin levels when compared with matched non-obese normoglycemic control subjects. Moreover, increased body weight in diabetes makes hypo-adiponectinemia more evident among overweight T2DM patients [26].

In a recent meta-analysis, [27] observed a significant inverse relationship between plasma adiponectin levels and the incidence of T2DM. Risk of T2DM appeared to decrease with increasing adiponectin levels. Also, low plasma adiponectin levels and negative association with insulin resistance in obesity and T2DM suggest that adiponectin

might have several therapeutic advantages [3]. Interestingly, low serum levels of adiponectin have been shown to independently predict future risk of developing T2DM [28].

Obesity is frequently associated with high plasma leptin levels in proportion to the degree of adiposity [26]. Most of the studies reported that serum leptin levels are correlated with obesity parameter and insulin resistance in obese subjects [29]. The mechanistic link between insulin resistance and hyperleptinism is not completely clarified. The highest concentration of leptin was found among obese type 2 diabetic patients, and a significant difference in leptin levels was discovered between obese diabetics and both control and diabetic groups. Our data confirmed that increased leptin levels in T2D patients were more related to the degree of adiposity than to the presence of T2D. Interestingly leptin was significantly associated with resistin in our population suggest that there could be existence of metabolic regulation in which both these factors are involved, consistent with the previous findings from other population [30].

Resistin has been proposed as an adipocyte secreted factor that is thought to link obesity and T2DM [8]. Its level was increased in the obese diabetic group than that in the non-obese diabetic group. The functions of resistin between glycemia, diabetes, insulin resistance, and obesity, are still argued. Results of current study commensurate in the normal range and did not show significant differences among the groups which reported that plasma resistin level did not differ between the groups as well as resistin does not appear to have an important link with insulin resistance and T2DM in human [31], [3]. In contrast, current results are not agreed with previous study, which found that T2DM subjects have significant higher resistin concentrations correlated linearly with BMI [32], also plasma resistin levels were higher in T2DM and obese subjects than in non-diabetic obese patients [33]. These results indicated the importance of taking these adipocytokines in consideration as biomarkers for T2DM and obesity during treatment. The potential role of serum adipokines as biomarkers in metabolic disorders cannot be ignored to prevent the future type 2 diabetes mellitus [3].

Diabetes mellitus is characterized by elevated level of oxidative stress indices, decreased levels of antioxidants defenses and lipid abnormalities due to lipid peroxidation.[10] Antioxidant vitamins and minerals are thought to be effective in increasing the activities of antioxidant defense enzymes, scavenging free radicals, preventing oxidative damage and thereby sparing lipid components of the cells against lipid peroxidation [11]. Oxidative stress is suggested to be a potential contributor to the development of diabetes mellitus and the associated complications [34]. This may be connected to the fact that the antioxidant status including antioxidant vitamins and minerals may be inadequate in diabetic subjects. The metabolic significance of the evaluation of antioxidant in diabetics is therefore of paramount importance. In addition to the antioxidant roles, they may act directly on glucose metabolism [25], [34].

The results of the current study indicate that serum vitamins A and E of the diabetic and obese diabetic groups are lower than the control group and this difference was statistically different in case of vitamin A. Increased oxidative stress in diabetic patients' results in higher utilization of these vitamins and consequently their deficiencies. Also, these antioxidant vitamins are excreted at higher than normal rates in patients with diabetes mellitus [35]. This may be connected to be hyperglycemia mediated polyuria in the patients; consequently, there is a decrease in the plasma levels of the vitamins (A & E) in these subjects. This may display the subject to further oxidative onslaught and decrease glucose tolerance leading eventually to the development of late complications of diabetes mellitus [25]. It may therefore be critical to suggest the inclusion of dietary supplementation of these antioxidant vitamins in the management of diabetes mellitus.

CONCLUSION

The importance of taking the adipocytokines in consideration as biomarkers for T2DM and obesity during treatment. Dietary supplementations of antioxidant vitamins (A&E) play essential in the management of type 2 diabetes mellitus.

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