Serum Visfatin in Relationt o Visceral Fat, Obesity and Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes mellitus is characterized mainly by hyperglycaemia occurring due to defects in insulin secretion, insulin action, or both. The International Diabetes Federation (IDF) reports that 463 million individuals worldwide were diagnosed with diabetes in 2019, and by 2040, that number is predicted to increase to 700 million. Aims and objectives: The aim of the study is to explore the role of adipokines and oxidative stress biomarkers in Type 2 diabetes mellitus (T2DM) patients of North Indian population. To investigate the anthropometric and clinical parameters (Insulin level, fasting plasma glucose, serum lipid profile, Liver function test, Kidney function test, HbA1c) in north Indian population having Type 2 Diabetes mellitus and Healthy controls.

Methods and materials: This case control study was conducted at Index Medical College, Hospital and Research Centre Indore MP, India. Either sex, age between 35 to 65 years patients with or without diabetes who reported in OPD of Medicine department during study period was enrolled in study. The study attempts to explore the role of adipokines and oxidative stress biomarkers in Type 2 diabetes mellitus (T2DM) patients of North Indian population.

Result: Kidney function profile (Creatinine and Urea) level were significantly higher in the case group in comparison to control group (P<0.05). Liver function test profile (AST and ALT) level were significantly higher in the case group in comparison to control group (P<0.05). but Albumin level was significantly lower in the case group in comparison to control group (P<0.05).

Conclusion: Lipid profile level (Total cholesterol, Triacylglycerol and HDL) were significantly higher in the case group in comparison to control group (P<0.05). Kidney function profile (Creatinine and Urea) level were significantly higher in the case group in comparison to control group (P<0.05).

Keywords: Liver function test, Kidney function test, Creatinine, Urea, Diabetes mellitus.

Introduction

Diabetes mellitus is characterized mainly by hyperglycaemia occurring due to defects in insulin secretion, insulin action, or both. The International Diabetes Federation (IDF) reports that 463 million individuals worldwide were diagnosed with diabetes in 2019, and by 2040, that number is predicted to increase to 700 million.

As a matter of fact, type 2 diabetes mellitus (T2DM) accounts for 4.2 million deaths worldwide in 2019 and is one of the main causes of illness and premature mortality worldwide. [1]

The condition is primarily prevalent in developing nations as well as industrialized areas (referred to as the "Occidental World"). It is largely caused by unhealthy lifestyle choices like high sugar and fat intake and inactivity.[2]

In Asia, the numbers of diabetics in China (>113.9 million diabetics), India (>62 million), and Malaysia (3.5 million) render the region a critical "hot spot" for diabetes. [3]

India is considered to be the "diabetic capital of the world". Diabetic condition is known to be associated with environmental, behavioral, and lifestyle factors such as a sedentary lifestyle and highly rich nutrition. In addition, the daily human-environment interactions and real-life activities that cause an individual's blood glucose to fluctuate remain relatively unexplored owing in part to data collection challenges.[4]

Need for the study: Type 2 diabetic mellitus patients with hyperglycemic crises have often been diabetic for several years, and tend to have adhered poorly to recommended insulin therapies.[5,6]

Material and Methods

This case control study was conducted at Index Medical College, Hospital and Research Centre Indore MP, India. Either sex, age between 35 to 65 years patients with or without diabetes who reported in OPD of Medicine department during study period was enrolled in study. The study attempts to explore the role of adipokines and oxidative stress biomarkers in Type 2 diabetes mellitus (T2DM) patients of North Indian population.

Study design - Case Control study

Place of Study - Index Medical College, Hospital and Research Centre Indore MP, India.

Selection of Participants: Subjects – Study subjects were divided into two groups:

A.   **Control group:** Control group included healthy volunteers with no history of Diabetes and any other systemic disease as Group I.

B.   **Study group:** Clinically diagnosed Type 2 Diabetes mellitus Patients as Group II

Sample Selection Criteria: Inclusion Criteria For study group

- Males and females between 35-65 years of age.

- For group I Type 2 diabetes diagnosed subjects but without any complication.

- The screening and management of patients as per American Diabetes Association guidelines**.**

For Control Groups:

- Healthy Males and females between 35-65 years of age.

- No prior history of Diabetes or any other systemic disease.

Exclusion criteria

- Individuals less than 35 years or greater than 65 years of age.

- Individuals suffering from disease like psychiatric disorders, hypertension, Alcoholics, Smokers, Pregnant and lactating women.

Sample Size:

1.   Control group (Group I): 160

2.   Type 2 Diabetes mellitus diagnosed Subjects (Group II): 160

Total Sample Size: 320 subjects

Methodology

Anthropometric measurements: Anthropometric measurements were made for each participant. Body weight was measured using an adult balance and standing height was measured to the nearest centimeter using a wall-mounted stadiometer without shoes prior to eating in the morning. Body mass index (BMI) values were determined by weight (kg) divided by height (m) squared. Waist circumference (WC) was directly measured on the skin midway between the mean point of iliac peak and the inferior border of the last rib at the level of the umbilicus while in a standing position at the end of gentle expiration. Hip circumference was measured over the widest part of the gluteal region at the level of pubic tubercle in standing position. Waist to Hip ratio were determined by WC (cm) divided by hip circumference (cm).

Blood sample collection

Sample collection from T2DM patients: About 4 mL of peripheral blood sample was collected in a red top tube (without any anticoagulant) from all T2DM patients. The serum was separated and one serum aliquot was immediately stored at −20 °C to −30 °C until further studies were conducted. Another serum aliquot was immediately sent for biochemical analyses.

Sample collection from healthy controls: Sample collections from all healthy age-matched control subjects were timed around routine blood draws that were part of the routine health checkup and, hence, did not require additional phlebotomy, and all participants were provided with a written informed consent form. About 4 mL of peripheral blood sample was collected in a red top tube (without any anticoagulant) for all controls. The serum was separated and one serum aliquot was immediately stored at −20 °C to −30 °C until further studies were conducted. Another serum aliquot was immediately sent for biochemical analyses.

Estimation of Biochemical parameters: Estimations of Insulin level, plasma glucose, serum lipid profile (Total cholesterol, High density lipoprotein-cholesterol and triglycerides), Liver function test (Total protein, albumin, total bilirubin, direct bilirubin, AST,ALT and ALP), Kidney function test (urea, creatinine, uric acid, calcium, phosphorus, sodium, potassium and chloride) was done using commercially available kits. HbA1c was estimated using HPLC based method.

Estimation of Oxidative Stress Biomarkers:

- ➤ **Estimation of Catalase:** (CAT) activity in lysate was determined spectrophotometrically by the method of Sinha *et al.*, 1972.

- ➤ **Estimation of Super oxide Dismutase (SOD):** SOD activity in lysate was determined spectrophotometrically by the method of Kakkar *et al.,* 1984.

- ➤ **Estimation Glutathione Peroxidase (Gpx):** GPx activity in lysate was determined spectrophotometrically by the method of Rotruck *et al*, 1973.

- ➤ **Estimation of Glutathione (GSH):** GSH activity in lysate was determined spectrophotometrically by the method of Kuo et *al.*, 1983.

- ➤ **Glutathione reductase**: Glutathione reductase activity in lysate was determined spectrophotometrically by the method of Hazelton and Lang *et al*, 1995.

- ➤ **Estimation of lipid peroxidation (LPO):** LPO activity as MDA in plasma was determined spectrophotometrically by the method of Stocks and Dormandy *et al*, 1971

- ➤ **Protein carbonyl group assay:** Protein carbonyl group activity in lysate was determined spectrophotometrically by the method of Levine and Williams et *al*, 1994.

Statistical analysis

Frequencies and percentages are used to report categorical variables, while the mean ± SD is used for continuous variables. With the Chi Square/Exact Fisher test, categorical variables were compared. We used the independent samples t-test to compare continuous variables. The normal distribution of the data was verified by testing each variable. The threshold of significant was set at $P < 0.05$.

Result

The present case-control study was conducted on Type 2 DM patients were selected who attending OPD, Index Medical College, Hospital and Research Centre Indore MP, India. A total 160 adult age group 35-65 years of both gender who were diagnosed Diabetic patients on the basis of WHO norms were enrolled in this study. 160 cases were similar age and sex normal healthy control. Diabetes profile (FBS, PPBS, HbA1c and duration of diabetes), Lipid biomarkers, Liver function test, kidney function test and oxidative stress were compared between diabetes and healthy control and find the association of serum levels of adipokines.

Table No. 1: Sex group distribution

Sex	Group		P value
	Case (n=160)	Control (n=160)	
Male	98 (61.3)	104 (65.0)	0.487
Female	62 (38.8)	56 (35.0)	

In case groups, 61.3% male and rest were female and in control groups 65.0% were male and 38.8% were female patients. By using the chi square test, we find insignificant distribution in both groups (P>0.05).

Table No. 2: Age group distribution

Age (Years)	Group		P value
	Case (n=160)	Control (n=160)	
≤50	71 (44.4)	81 (50.6)	0.262
> 50	89 (55.6)	79 (49.4)	

We found that the statistically insignificant higher older age population in case group distribution in compare to control group (P>0.05).

Table No. 3: Anthropometric variables

	Group		P value
	Case (n=160)	Control (n=160)	
Age (Years)	50.46±8.69	49.94±7.90	0.575
Weight (kg)	74.15±10.47	71.61±10.28	**<0.001**
Height (cm)	162.02±7.47	162.94±6.89	0.253
Body Mass Index (kg/m²)	28.36±4.17	25.35±4.11	**<0.001**
Waist Circumference (cm)	89.01±5.91	84.18±4.92	**<0.001**
HIP Circumference (cm)	99.35±5.98	101.98±7.06	**0.004**
Waist Hip Ratio	0.92±0.06	0.86±0.04	**<0.001**

Above table shows the anthropometric distribution of the studied patients; we observed the statistically significant higher weight, BMI, Waist Circumference and Waist Hip Ratio in case group in compare to control group (P>0.05). while HIP Circumference was significant lower in case group. But age and height were insignificant deference in both groups.

Table No. 4: Blood Glucose level in studied patients

Blood Glucose level	Group		P value
	Case (n=160)	Control (n=160)	
Fasting Plasma Glucose	204.54±43.77	85.44±15.66	**<0.001**
Insulin level	41.11±10.74	10.0±3.39	**<0.001**
Glycated Haemoglobin (HbA1c)	11.68±2.23	5.36±0.28	**<0.001**

Blood Sugar level (Fasting Plasma Glucose, Insulin level and HbA1c) were significantly higher in the case group in comparison to control group (P<0.001).

Table No. 5: Lipid profile in studied patients

	Group		P value
	Case (n=160)	Control (n=160)	
Total cholesterol (mg/dl)	203.65±49.63	133.41±25.95	**<0.001**
Triacylglycerol (mg/dl)	193.05±66.09	107.1±28.61	**<0.001**
HDL cholesterol (mg/dl)	33.88±4.09	53.63±4.74	**<0.001**

Lipid profile level (Total cholesterol, Triacylglycerol and HDL) was significantly higher in the case group in comparison to control group (P<0.05).

Table No. 6: Kidney function profile in studied patients

	Group		P value
	Case (n=160)	Control (n=160)	
Creatinine (mg/dl)	1.32±0.21	0.88±0.18	**<0.001**
Urea (mg/dl)	31.88±7.67	27.59±7.91	**0.009**

Kidney function profile (Creatinine and Urea) level were significantly higher in the case group in comparison to control group (P<0.05).

Liver function test profile (AST and ALT) level were significantly higher in the case group in comparison to control group (P<0.05). but Albumin level was significantly lower in the case group in comparison to control group (P<0.05).

Table No. 7: Oxidative stress markers

Oxidative stress markers	Group		P value
	Case (n=160)	Control (n=160)	
Malondialdehyde (MDA)	3.65±1.62	1.32±0.45	**<0.001**
Catalase	30.42±13.75	48.00±22.84	**<0.001**
Superoxide dismutase (SOD)	2.32±0.26	4.86±0.25	**<0.001**
Glutathine reductase	37.10±7.86	63.23±12.74	**<0.001**

Above table shows that the oxidative stress markers Malondialdehyde (MDA) level were significantly higher in the case group in comparison to control group (P<0.05). But catalase, SOD and Glutathine reductase level was significantly lower in the case group in comparison to control group (P<0.05).

Discussion

Adiponectin is an adipokine secreted specifically from the adipose tissue. The inverse relationship between body fat and serum adiponectin levels has been demonstrated, and weight reduction can increase adiponectin levels.[7] Adiponectin modulates glucose metabolism by having insulin-sensitising effects. Adiponectin also decreases circulating free fatty acid concentrations and muscle triglyceride content by stimulating fatty acid oxidation in muscle via AMP-activated protein kinase (AMPK).[8] Thus, adiponectin is a hormone that links adipose tissue and whole-body glucose metabolism. Low adiponectin levels have been associated with type 2 diabetes and insulin resistance.[9] Adiponectin has been found to have vaso-protective and anti-inflammatory effects and therefore could be viewed as a potential link between MS and its cardiovascular consequences. The current study we tried to explore the role of adipokines and oxidative stress biomarkers in Type 2 diabetes mellitus (T2DM) patients of North Indian population. find the correlation of Adiponectin levels in metabolic syndrome and also with various components of metabolic syndrome. A special interest was to correlate adipokines (Visfatin and Adeponectin), oxidative stress levels, anthropometric and clinical parameters in T2DM patients.

Sharma R et al[10], Weyer C et al[11], and **Amita Yadav et al[12]** did case-control studies of the adiponectin levels and its association with blood sugar level. The case and control study were not adopted by us because it was very difficult to convince healthy and mentally fit patients to take part in the study and to go through several tests and screening.

As per WHO criteria; T2DM is characterized by elevated fasting blood sugar (FBS) (≥126mg/dl) or post-prandial blood sugar (PPBS) (≥200mg/dl) concentrations.[13] The American Diabetes Association (ADA) Standards of Medical Care in Diabetes added that glycated haemoglobin (HbA1c) as an important standard for the diagnosis of pre-diabetes and diabetes (5.7-6.4% and ≥6.5%, respectively).[14]

The Present study noted that in case groups, 61.3% male and rest were female and in control groups 65.0% were male and 35.0% were female patients. We found that the statistically insignificant higher older age population in case group distribution in compare to control group (P>0.05). Present study noted the statistically significant higher weight, BMI, Waist Circumference and Waist Hip Ratio in case group in compare to control group (P>0.05). while HIP Circumference was significant lower in case group. But age and height were insignificant deference in both case and control groups. Blood Sugar level (Fasting Plasma Glucose, Insulin level and HbA1c) were significantly higher in the case group in comparison to control group (P<0.001). Lipid profile level (Total cholesterol, Triacylglycerol and HDL) were significantly higher in the case group in comparison to control group (P<0.05). Kidney function profile (Creatinine and Urea) level were significantly higher in the case group in comparison to control group (P<0.05). Liver function test profile (AST and ALT) level were significantly higher in the case group in comparison to control group (P<0.05). but Albumin level was significantly lower in the case group in comparison to control group (P<0.05).

Limitations

➤ This was a cross-sectional study which does not allow for conclusions regarding causality.

➤ This study included middle-aged individuals, without prevalent cardiovascular disease. Caution is thus needed in the extrapolation of the findings to other populations, i.e. younger, of other ethnicity or with cardiovascular disease. Moreover, metabolic or hypertension were less prevalent than type 2 diabetes mellitus and impaired fasting glucose in the study population.

➤ Finally, the adipokines measured are only a small fraction of the wide array of pro- and anti-inflammatory biochemical indices that are produced by the adipose tissue.

Recommendations:

Because of the epidemic of obesity and a sedentary lifestyle worldwide, diabetes is flattering progressively usually predictable. Development of a method for suitable estimate of diabetes in regular clinical practice offerings a main challenge for physicians and public health policy makers. The present study provided suggestion of the effectiveness for assessment of serum adiponectin level as an appropriate and sensitive biomarker for the estimation of diabetes and inflammation. Prospective and population-based studies along with interventions to increase adiponectin level are however required to confirm the associations.

Conclusion:

❖ In case groups, 61.3% male and rest were female and in control groups 65.0% were male and 35.0% were female patients.

❖ Age and height were insignificant deference in both groups.

❖ The statistically significant higher weight, BMI, Waist Circumference and Waist Hip Ratio in case group in compare to control group (P>0.05).

❖ HIP Circumference was significant lower in case group.

❖ Blood Sugar level (Fasting Plasma Glucose, Insulin level and HbA1c) were significantly higher in the case group in comparison to control group (P<0.001).

❖ Lipid profile level (Total cholesterol, Triacylglycerol and HDL) were significantly higher in the case group in comparison to control group (P<0.05).

❖ Kidney function profile (Creatinine and Urea) level were significantly higher in the case group in comparison to control group (P<0.05).

❖ Liver function test profile (AST and ALT) level were significantly higher in the case group in comparison to control group (P<0.05).

❖ Albumin level was significantly lower in the case group in comparison to control group (P<0.05).

❖ the oxidative stress markers Malondialdehyde (MDA) level were significantly higher in the case group in comparison to control group (P<0.05).

❖ Catalase, SOD and Glutathine reductase level was significantly lower in the case group in comparison to control group (P<0.05).

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