



## PLASMA LEPTIN AND ADIPONECTIN IN PATIENTS WITH MICRO-VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS

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### ABSTRACT

**Introduction** Diabetes mellitus is a chronic metabolic disorder characterised by hyperglycemia resulting from absolute or relative insulin deficiency. Uncontrolled diabetes leads to microvascular complications like diabetic nephropathy, retinopathy, neuropathy etc. There are many markers which help in the detection of diabetes mellitus. Leptin and adiponectin are 2 such important markers which has a role in the metabolism of glucose as well as in the inflammation process. Leptin deficiency results in hyperphagia, obesity and diabetes mellitus. Adiponectin is primarily secreted by adipose tissue and is has anti-diabetic, and anti-atherogenic properties. **Aim of the study** This study aims to detect the association between the plasma concentration of leptin and adiponectin in diabetic patients with or without micro-vascular complications. **Materials and methods** Diabetic patients with micro-vascular complications are included in this comparative study as group I, while group II includes diabetic patients without micro-vascular complications. Serum levels of leptin and adiponectin were assessed in both the groups along with glycaemic status (fasting blood sugar, post prandial blood sugar and glycated haemoglobin). These parameters were compared between both groups using a t-test. **Results** The diabetic patients with microvascular complications are having high serum levels of leptin and low levels of adiponectin when compared with the patients without microvascular complications. **Conclusion** This study confirms that serum levels of leptin have a positive correlation while serum levels of adiponectin have a negative correlation in diabetic patients with microvascular complications when compared to diabetic patients without microvascular complications.

**KEYWORDS :** Leptin, Adiponectin, Microvascular complications, Type 2 diabetes.

### INTRODUCTION

Diabetic mellitus is one of the most common metabolic disorder associated with or without micro vascular complications. Incidence of diabetes is now increasing in both developing as well as in developed nations. The development of type 2 DM in individuals are usually preceded by a pre-diabetes stage, in which there will be impaired glucose tolerance (IGT) and impaired fasting glucose (IFG).<sup>(1,2)</sup>

Micro vascular complications of type 2 diabetes mellitus usually develop insidiously with few symptoms, but in the final stages, irreversible changes can occur (end stage renal disease, loss of vision etc). The two important reversible risk factors for the development and progression of microvascular complication like nephropathy and retinopathy are the elevated blood glucose and blood pressure levels.<sup>(3,4)</sup> Insulin resistance is closely associated with abdominal adiposity, a surrogate measure of visceral adiposity.<sup>(5)</sup> Adipocytes secrete a variety of bioactive substances known as adipokines which includes leptin and adiponectin.

Leptin is mainly secreted by white adipose tissue and plays a major role in regulating energy balance and body weight.<sup>(6)</sup> Leptin is a protein that circulates in proportion with body fat mass, provides information about nutritional status and subcutaneous fat mass to neural centers that regulate feeding behaviour, appetite, and energy expenditure.<sup>(7)</sup> Serum leptin concentration is directly related to the content of adipose tissue.<sup>(8,9,10)</sup> Leptin level increases in the postprandial period and decreases during starvation.<sup>(11,12)</sup> Leptin plays a crucial role in the regulation of energy homeostasis, insulin action and lipid metabolism<sup>(13)</sup>. Leptin concentration reflects fat mass and also serves as an important signal of body energy stores. Plasma leptin is positively related to insulin resistance and adiposity.<sup>(14,15)</sup>

Serum leptin levels are a risk factor for decreased renal function. But some studies explain that there was no difference

in leptin levels in diabetic patients with or without kidney disease.<sup>(16)</sup> In some studies, patients with diabetic kidney disease have higher serum leptin levels than those without diabetic kidney disease.<sup>(16,17)</sup> Leptin administration in patients with generalized lipodystrophy shows a dramatic improvement in albuminuria and metabolic parameters.<sup>(18,19)</sup> In type 2 diabetes, there is a significant positive correlation between metabolic derangements and microangiopathic complications (retinopathy).<sup>(20,21)</sup> Leptin has an anti-inflammatory role which result in endothelial dysfunction, vascular inflammation and proliferation of vascular smooth muscle cells. Vascular inflammation is the main pathological mechanism of diabetic microvascular complications.<sup>(22)</sup>

Adiponectin is a hormone synthesized mainly in the white adipose tissue and also in the brown adipose tissue, skeletal muscle, liver, colon, cardiac muscle etc. The normal concentration of plasma adiponectin ranges between 5 to 30 µg/mL. Women have high adiponectin concentrations when compared to men.<sup>(23,24)</sup> Adiponectin possesses anti-inflammatory and anti-atherogenic effects. Adiponectin increases the insulin sensitivity and tissue fat oxidation which results in reduction in free fatty acid levels. Adiponectin might reduce the risk of type 2 diabetes by many ways which includes suppression of gluconeogenesis, improved fatty acid oxidation in liver, stimulate glucose uptake in skeletal muscle, stimulation of insulin secretion etc.<sup>(25)</sup> Adiponectin levels decreases with body fat percentage, central fat distribution, fasting plasma insulin, and oral glucose tolerance. Adiponectin levels are lower in patients with coronary artery disease suggesting a possible correlation of decreased adiponectin level in vasculopathic states.<sup>(26,27)</sup>

There is strong evidence between the level of adiponectin and risk for diabetes. Plasma adiponectin levels are inversely related to insulin resistance, obesity, type 2 diabetes, macrovascular complications, coronary artery disease etc.<sup>(28,29)</sup>

It also affects the development or progression of diabetic microvascular complications. The relationship between serum adiponectin level and microvascular complications are controversial.<sup>31</sup> The relation of adiponectin with diabetic retinopathy is still not well known. In some studies, it was found that the level of adiponectin has no relationship with diabetic retinopathy.<sup>(30)</sup> In a study conducted by Chan.Hee Jung et al, it was found that the levels of adipocytokines were different depending upon the type of microangiopathies.<sup>(31)</sup>

So, we decided to conduct a research study to assess the relationship between plasma concentration markers in diabetes mellitus (leptin and adiponectin) in patients with or without micro-vascular complications coming from different parts of Malabar region of Kerala.

## MATERIALS AND METHODS

**Study setting:** Departments of Medicine, Physical Medicine and Rehabilitation & Biochemistry - Govt. Medical College, Kozhikode, Kerala - a tertiary care teaching hospital.

**Study design:** Comparative cross-sectional study.

### Inclusion criteria

- 1) Diabetic patients with micro-vascular complications aged between 20 - 60 years and similar age and gender matched group without microvascular complications.
- 2) Those who are willing to give valid informed consent.

### Exclusion criteria

- 1) Seriously ill patients
- 2) Pregnancy
- 3) Lactating mother

### METHOD:

Diabetes patients in the age group between 20 - 60 years (both gender) with and without microvascular complications, attending the outpatient Departments of Medicine and Physical Medicine and Rehabilitation, Govt. Medical College are included in this study.

**Sample size:** 180 patients

**Study period:** 2 years

### Study variables:

- 1) Fasting blood sugar
- 2) Post prandial blood sugar
- 3) HbA1c
- 4) Fasting Plasma Leptin
- 5) Fasting Plasma Adiponectin

Patient's details were collected in the proforma, which included name, age, gender, address, contact details, occupation, history of type 2 diabetes including its duration, treatment, and any other co-morbidities. Anthropometric measurements like weight and height were measured.

### Laboratory investigations

Venous blood samples (5-7 ml) were collected from all patients in a vacutainer after a fasting for at least 10 hours. Samples were collected between 6 and 8 am and then post prandial blood samples were also collected. The following parameters were estimated in both groups I and II. Fasting blood sugar (FBS), post prandial blood sugar (PPBS), HbA1c (glycated hemoglobin), leptin and adiponectin.

Fasting blood sugar and post prandial blood sugar (PPBS) were estimated by the glucose oxidase peroxidase method. HbA1c was estimated by HPLC (high-performance liquid chromatography) based automated analyzer.

Leptin and adiponectin were estimated after the serum was

separated by centrifuging the coagulated blood samples at 4000 rpm for 5 minutes at 4 °C, and stored at -80 °C until the day of measurement. The serum leptin and adiponectin concentration was measured using a commercially available human enzyme-linked immunoassay (ELISA) kit. The principle of the ELISA is a two-step sandwich enzyme immunoassay. The assay makes use of two highly specific monoclonal antibodies.

### Statistical analysis

Statistical analysis was done using SPSS -18 soft ware.

## RESULTS

Total patients included in our study was 160. Of these, 80 were with and other 80 without complications of diabetes mellitus. They are included as group I (with complications) and group II (without complications). In the group I, there were 34 males (42.5 %) and 46 females (57.5 %). In the group II there were 36 males (45 %) and 44 females (55%). In the group I mean age of participants in our study were  $54 \pm 6$  years. Among the participants in group II, the mean age group was  $51 \pm 5$  years. In the group I, the mean fasting blood sugar levels were found to be  $181.32 \pm 35.56$  and the mean post prandial blood sugar level was  $293 \pm 25.24$ . In the group II, mean fasting blood sugar levels was  $164.68 \pm 20.32$  and the mean post prandial blood sugar level was  $233 \pm 19.26$ .

Duration of diabetes less than 5 years in our study was 60 (37.5%) and more than 5 years was 100 (62.5%). Mean HbA1c levels among group I were  $8.63 \pm 0.74$  where as in the group II the levels were  $7.98 \pm 0.83$ .

The serum leptin levels (ng/ml) in the group I patients with microvascular complications were  $26.39 \pm 8.23$ . In group II patients without microvascular complications the mean value of serum leptin was  $19.42 \pm 6.89$ . In the group I patients with microvascular complications the serum adiponectin levels ( $\mu\text{g/mL}$ ) was 5.1, while in the group II patients who were having no microvascular complications, the level was 7.3.

## DISCUSSION

Our study aims to compare the significance of serum levels of leptin and adiponectin in diabetic patients with and without microvascular complications. 50% of the diabetic patients were with microvascular complications and other 50% of the patients were without microvascular complications.

In our study, the diabetic patients in the group I had mean fasting blood glucose  $181.32 \pm 35.56$ . In the group II, mean fasting blood sugar levels were  $164.68 \pm 20.32$ . In a study conducted by Li-Kun Wang, et al in 2019 in type 2 diabetes patients with microvascular complications mean fasting blood glucose level were 158.58 mg/dL, while in patients without microvascular complications were 144.35 mg/dL.<sup>32</sup> The mean fasting blood sugar level in this study when compared to our study is less.

In our study, 62.5% patients had duration of type 2 diabetes mellitus more than 5 years. In a study conducted by Walaa Mohammed Saeed et al in 2023, the mean duration of diabetes was  $12.5 \pm 5.2$ .<sup>34</sup>

Mean HbA1c levels in group I of our study was  $8.63 \pm 0.74$  while in the group II the levels were  $7.98 \pm 0.83$ . Similar study done by Kazutaka Nozawa et al in 2022 the Mean HbA1c levels was found to be  $7.2 \pm 1.0$  in diabetic patients with peripheral neuropathy where as in the diabetic patients without peripheral neuropathy in the levels were  $6.9 \pm 1.1$ . So, the mean HbA1c levels in this study when compared to our study is found to be low.<sup>(32)</sup>

In our study, the serum leptin levels (ng/ml) was  $26.39 \pm 8.23$  in group I patients with microvascular complications. In the

group II patients without microvascular complications the mean value of serum leptin was  $16.41 \pm 7.32$ . In a study conducted by Talaat A Abdel Aaty et al in 2020 the mean value of serum leptin was  $28.05 \pm 8.01$  in diabetic patients with microvascular complications. In the diabetic patients without microvascular complication the serum leptin level was found to be  $18.10 \pm 6.14$ .<sup>(22)</sup>

In group I diabetic patients with microvascular complications in our study the serum adiponectin levels ( $\mu\text{g/mL}$ ) was  $2.83 \pm 0.56$ , while in the group II patients who are without microvascular complications the adiponectin level were  $3.93 \pm 0.46$ . In a study conducted by Li-Kun Wang, et al in 2019 the the mean value of serum adiponectin value in diabetes with microvascular complications were  $1.61 \pm 0.12$ , while in the diabetic patients without microvascular complications it was  $3.57 \pm 0.11$ .<sup>(33)</sup>

In some other studies, the serum adiponectin levels in diabetic patients with microvascular complication were higher when compared to the patients without microvascular complication.<sup>(34)</sup> So the mean adiponectin levels in this study when compared to our study is comparable.

### Limitations

1. Single institution
2. Small patient size

### CONCLUSION

This study confirms that serum levels of leptin have a positive correlation while serum levels of adiponectin have a negative correlation in diabetic patients with microvascular complications when compared to diabetic patients without microvascular complications.

### Acknowledgment

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### Ethical Considerations

The invasive procedure done in this study group is aspiration of 5 ml blood. No drugs were used in the study.

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### Conflict of Interest

There are no conflicts of interest.

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