

Association of Resistin, Visfatin, and Osteoprotegerin in Diabetes Mellitus

Ghadah A. Aljebori¹ Saad M. Hussein¹ Monem M. Alshok²

1. Department Of Physiology, College of Medicine, Babylon University/Iraqi

2. Department Of Medicine, College of Medicine, Babylon University/Iraqi

Abstract

The aims of the study to evaluate the association between diabetes mellitus and resistin, visfatin and osteoprotegerin levels and studying the interaction of resistin, visfatin and osteoprotegerin with duration of diabetes mellitus. This is a case/control study conducted on a total 88 subjects; sixty eight patients (25 male and 43 female) compared to twenty healthy persons (7 males and 13 females). Venous blood collected from the patients attending to Diabetic Center in Merjan Teaching Hospital / Babylon/ Iraq. Serum was prepared from collected blood, and used to investigate resistin, visfatin and osteoprotegerin. The present study findings revealed a significant decrease in resistin, visfatin and osteoprotegerin levels when compared with controls. On other the hand, the study showed that the visfatin and osteoprotegerin increased in 11-15 years of duration of diagnosis, while there was no relation between resistin and duration of diagnosis.

Keywords: Diabetes Mellitus, resistin, visfatin and osteoprotegerin.

Introduction:

Diabetes mellitus is known as a metabolic disorder characterized by chronic excessive blood glucose (Hyperglycemia), which may be due to a defect in insulin secretion or in the effectiveness of insulin, or both. Diabetes Mellitus (DM) is continuing to become a health problem since the prevalence of DM has increased dramatically over the past two decades (**Detels, et al., 2006; Susanti, et al., 2010 and ADA, 2011**). The following symptoms may be associated with acute or chronic hyperglycemia, with the first three comprising the classic hyperglycaemic triad polyphagia, polydipsia, polyuria, blurred vision, fatigue, weight loss and impotence (male) (**Nikolic and Jovanovic, 2012**).

The following classification system identifies six types of diabetes mellitus: Insulin-dependent (IDDM) and non-insulin dependent diabetes (NIDDM) will be renamed type 1 and type 2 diabetes (**Saleh, 2011**).

- Type 1 diabetes mellitus; (β -cell destruction, usually leading to absolute insulin deficiency).
- Type 2 diabetes mellitus; (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance).
- Type 3 diabetes mellitus
- Type 1.5 diabetes mellitus or Latent Autoimmune Diabetes of Adulthood.
- Maturity Onset Diabetes of Young (MODY)
- Gestational diabetes mellitus

Some studies found that there are some cytokines related with diabetes mellitus, one of them is resistin, a cysteine-rich polypeptide with 108 amino acid residues synthesized and secreted by adipocytes, immune and endothelial cells (**Park and Ahima, 2013**). There is strong correlations between resistin and obesity, serum resistin levels will increase with increased adiposity (**Lee, et al., 2005**). Visfatin is preferentially expressed in visceral adipose tissue and possessed insulin-mimetic bioactivity (**El-Mesallamy, et al., 2011**). Osteoprotegerin (OPG) is a secreted glycoprotein belonging to the tumor necrosis factor receptor super family. It is mainly secreted by bone but is also secreted by a variety of different tissues including endothelial and smooth muscle cells (**Nabipour, et al., 2010**).

Resistin is produced mainly by white and brown adipose and in several other tissues, including the hypothalamus, pituitary and adrenal glands, pancreas, gastrointestinal tract, myocytes, spleen and white blood cells. Resistin antagonizes insulin action, and it is down regulated by rosiglitazone and peroxisome proliferator-activated receptor agonists (**Gerstmayr, et al., 2003; Liu, et al., 2011**). Its expression is predominantly localized in macrophages and stromal cells of adipose tissue rather than adipocytes (**Bohler, et al., 2010; Schwartz and Lazar, 2011**). The adipocytokine resistin which belongs to a family of cysteine-rich C-terminal proteins known as resistin-like molecules (RELM; RELM alpha/FIZZ 1 and RELM beta/FIZZ 2) of FIZZ (found in inflammatory zone) are thought to be involved in inflammatory processes (**Steppan, 2001**). (**Gharibeh, et al., 2010**) suggest that resistin plays a role in the pathogenesis of obesity and insulin resistance, indirectly, contribute to the development of type 2 diabetes.

Visfatin is a peptide that is predominantly expressed in, and secreted from, visceral adipose tissue (**Fukuhara, et al., 2005; Hug and Lodish, 2005**). Visfatin has an expression in hepatocytes and muscles (**Cosford, et al., 2010; Garten, et al., 2010**). Visfatin was previously identified as a protein involved in β -cell maturation (pre- β -cell colony- enhancing factor) (**Alghasham and Barakat, 2008 and Kaminska, et al., 2010**). Visfatin

displays proinflammatory characteristics and modulated immune functions and exerts insulin-mimicking effects through activation of an insulin receptor, although in a manner distinct from that of insulin (**Fukuhara, et al., 2005; Jacques, et al., 2012**). Visfatin has a role in multiple aspects of pancreatic β -cell biology, including a role in the regulation of insulin secretion and receptor signalling. This confirm the previously reported modulatory role of visfatin in the insulin signalling pathway, and suggest that these actions occur via its ability to synthesize nicotinamide mononucleotide (NMN), a precursor for the metabolic co-factor nicotinamide adenine dinucleotide (NAD⁺) (**Brown, et al., 2010**). Visfatin is down-regulated by overfeeding. Under physiologic conditions, visfatin does not appear to control glucose metabolism but may play a regulatory role in lipid metabolism (**Sun, et al., 2007**). Increased body weight is tightly associated with insulin resistance and type 2 Diabetes Mellitus. Adiponectin and visfatin levels can be associated with insulin sensitivity at obese diabetic patients compared to healthy control (**Kara, et al., 2014**).

Osteoprotegerin (OPG) is present in connective tissues, especially in vasculature, in the arterial wall, OPG also circulates in blood, although the concentrations here are considerably lower than in tissue (**Olesen, et al., 2005**). The cellular source of OPG in bone is considered to be osteoblasts, whereas in the vasculature it is ascribed to vascular smooth muscle cells (VSMC), since endothelial cells produce only smaller amounts (**Nybo and Rasmussen, 2008**). Studies in vitro and in animal models suggest that OPG inhibits vascular calcification, in addition to inhibiting apoptotic passive calcification, the ability of OPG to inhibit alkaline phosphatase-mediated osteogenic differentiation of vascular cells is also likely to contribute to the protective role of OPG (**Van Campenhout and Golledge, 2009**). The accumulation of OPG may be a part of the generalized matrix changes in the arterial wall in diabetes (**Chung, et al., 2008**). Serum OPG was found associated with carotid intima media thickness in women with previous gestational diabetes (**Akinci, et al., 2008**). Several studies have suggested plasma OPG as a predictor of cardiovascular disease (**Kiechl, et al., 2004; Ueland, et al., 2004 and Nybo and Rasmussen, 2008**). Increased osteoprotegerin plasma levels have been reported in type2 diabetic patients, and the increased osteoprotegerin levels were associated with microvascular complications (**Knudsen, et al., 2003**). Osteoprotegerin is associated with glycaemic control and cardiovascular diseases in patients with type 1 diabetes, compatible with the hypothesis that OPG is associated with the development of diabetic vascular complications (**Rasmussen, et al., 2006**). Serum OPG levels were higher in diabetic patients suffering from myocardial infarction, so this parameter could be a risk marker for MI in diabetic patients (**Hussain, et al., 2014**).

Materials and Methods:

Materials

Subjects:

This a case/ control study was carried out through the period from October, 2014 to April, 2015, in Marjan Teaching Hospital / Babylon/ Iraq. All samples were randomly selected from the patients attending the Diabetic Consultation Unit at the Hospital. The study was carried out on 68 diabetic patients (25 male and 43 female) and 20 apparently healthy subjects (7 males and 13 females). Every patients had been asked about name, age, onset of disease, duration of disease and the type of diabetes mellitus.

Blood sampling:

Five milliliters of venous blood sample were drawn from each patients by vein puncture and poured slowly into plain tubes. The same quantity of blood was collected from control subjects, the sample were left at room temperature for thirty minutes to two hours for clotting, then centrifugation of samples were done for 10-15 minutes at 1000*g, and then serum were separated into several parts:

Aliquot of serum is transfer into 1 ml Eppendorf tubes, which was used to measure resistin, visfatin and osteoprotegerin. The tubes were stored at -20 centigrade until analysis.

Methods

Blood tests:

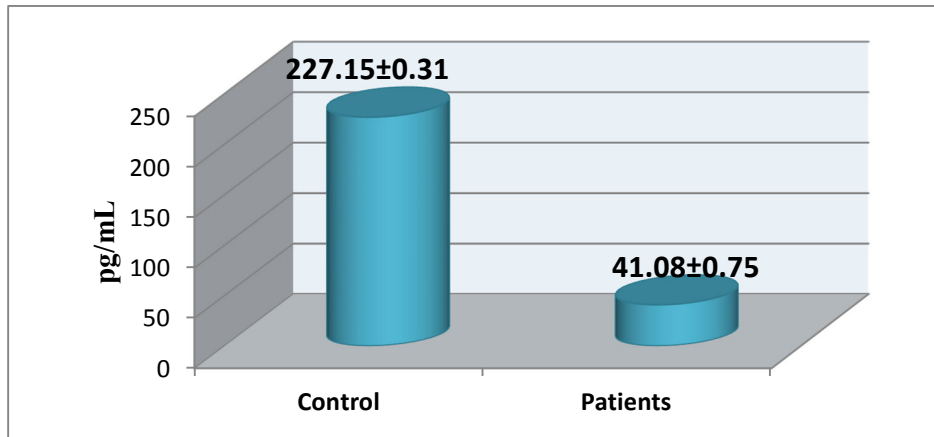
Resistin, visfatin and osteoprotegerin levels measured by ELISA kits.

Statistical analysis:

Statistical analysis was performed by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The normal distribution was conformed correlation analysis, independent t-test was used to estimate differences between two groups in continuous variables. Results are reported as mean and standard deviation (mean \pm SD) unless otherwise indicated. A p-value of ≤ 0.05 was considered as a lowest limit of significant (**Danial, 1999**).

RESULTS:

Resistin Level: there is significant difference in level of resistin between diabetic patients and controls ($P < 0.05$), the resistin level in patients was 41.08 pg/mL while in controls it was 227.15 pg/mL. (Figure1).



- Values are mean ±SD.

Fig (1): Resistin Level in Diabetic Patients and Control Groups.

According to duration of diagnosis, we measured resistin level but did not find any significant difference ($P > 0.05$) (Figure 2).

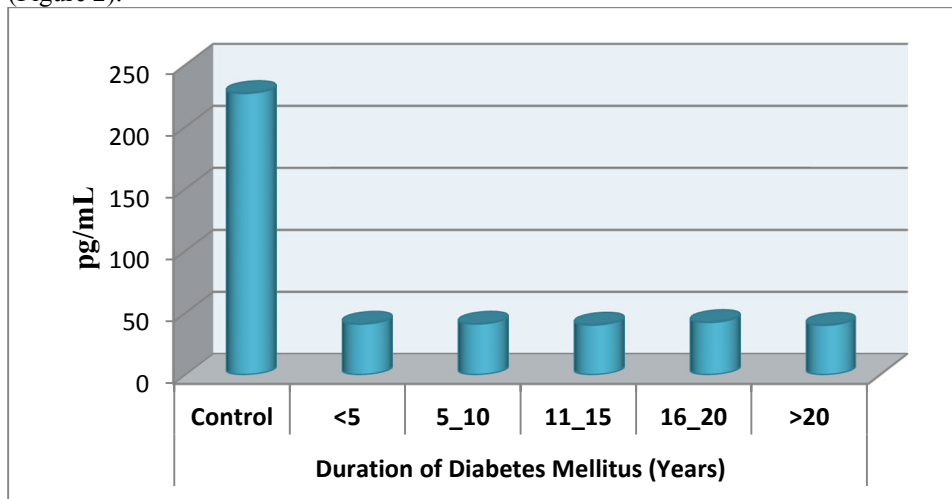
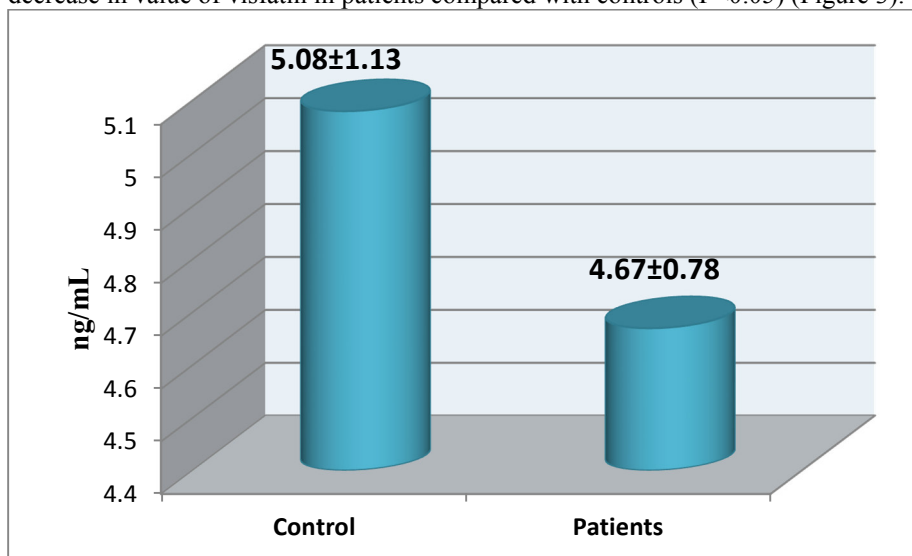


Fig (2): Resistin Level in Patients with Diabetes According to Duration of Disease.

Visfatin level: results of visfatin were similar to the results of resistin where we found that there is significant decrease in value of visfatin in patients compared with controls ($P < 0.05$) (Figure 3).



- Values are mean ±SD.

Fig (3): Visfatin Level in Diabetic Patients and Control Groups.

Unlike to resistin, there was significant difference in level of visfatin between groups of duration of diagnosis, the results are as follows (Figure 4):

- _ In <5 years of diagnosis, visfatin level was 3.03 ng/mL.
- _ In 5-10 years of diagnosis, visfatin level was 4.51 ng/mL.
- _ In 11-15 years of diagnosis, visfatin level was 7.56 ng/mL.
- _ In 16-20 years of diagnosis, visfatin level was 5.51 ng/mL.
- _ In age above 20 years of diagnosis, visfatin level was 6.36 ng/mL.

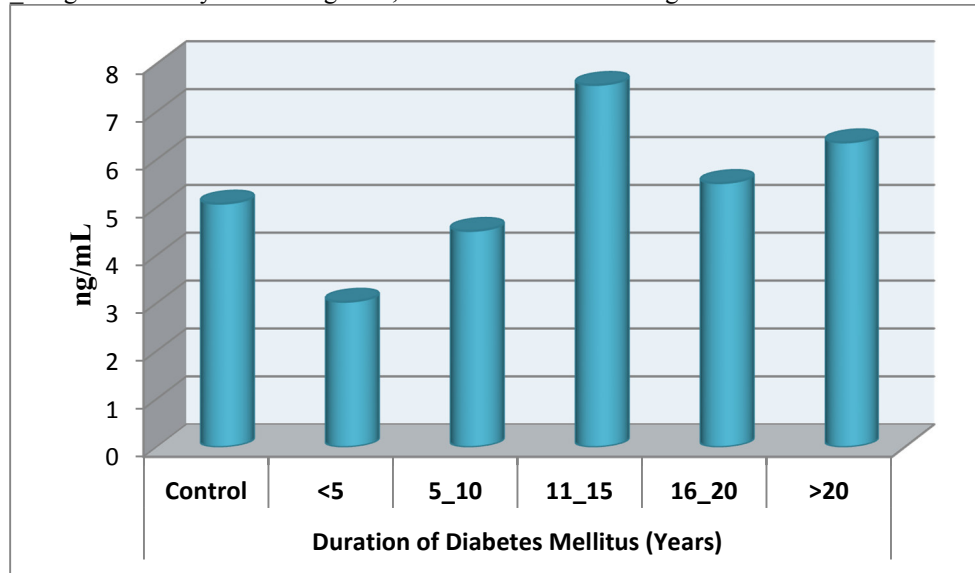
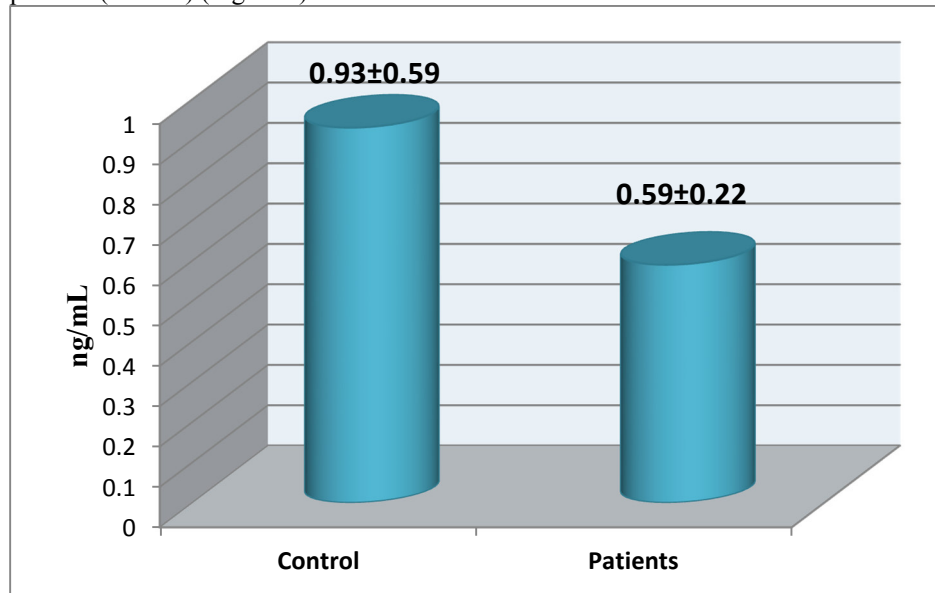


Fig (4): Visfatin Level in Patients with Diabetes According to Duration of Disease.

Osteoprotegerin level: as in resistin and visfatin, the level of osteoprotegerin decrease significantly in diabetic patients ($P < 0.05$) (Figure 5).



- Values are mean \pm SD.

Fig (5): Osteoprotegerin Level in Diabetic Patients and Control Groups.

Osteoprotegerin level increase in 11-15 years of duration of diagnosis which is 1.42 ng/mL, at less than 5 years it was 0.63 ng/mL, at 5-10 years it was 0.38 ng/mL while in 16-20 and for more than 20 years there is no significant difference (Figure 6).

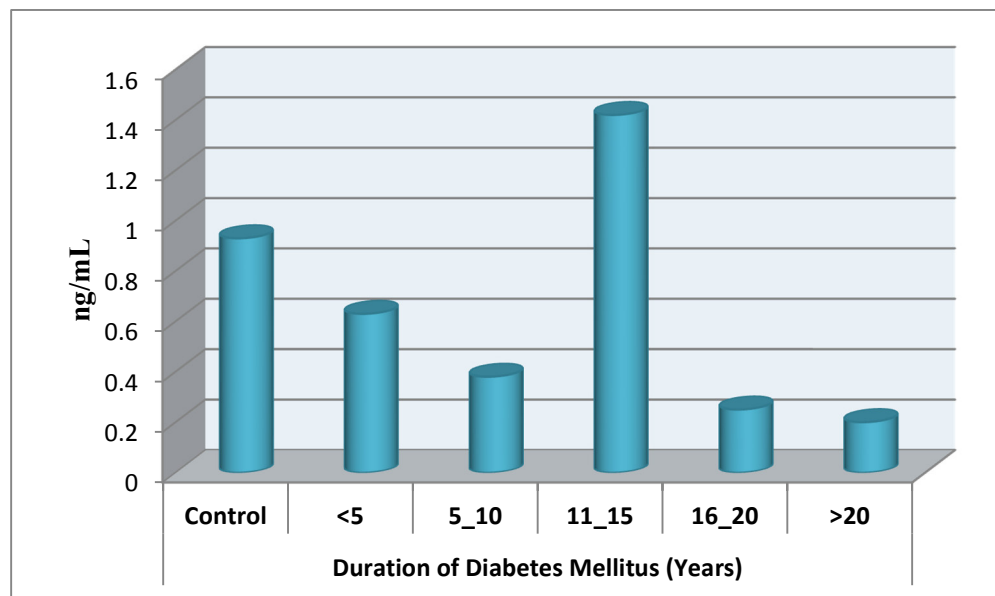


Fig (6): Osteoprotegerin Level in Patients with Diabetes According to Duration of Disease.

Discussion:

The results of our study show that the resistin, visfatin and osteoprotegerin are lower in diabetic patients than control group (Figures 1; 2; 3) respectively (Moller,2000 and Al-Ani, *et al.*,2010). According to duration of diagnosis, this study found that there is no relation between resistin and duration of diagnosis this result agree with (Al-Ani, *et al.*,2010), while during 11-15 years of diagnosis the visfatin and osteoprotegerin are high. Resistin reduces glucose uptake by adipocytes and skeletal muscle and reduces insulin tolerance. In patients with diabetes mellitus type 1 was hyperinsulinemia due to insulin injection and resistin antagonizes insulin action lead to decrease serum resistin levels in compared to controls. Our result agrees with Fehmann, *et al.*,(2002) and Schaffler, *et al.*,(2004).

Kara, *et al.*(2014) found that the level of visfatin of obese T2DM patients were increased as compared to that in control group but in non obese and control group was not different ($P>0.05$). Esteghamati , *et al.*,(2011) and Ahmed , *et al.*,(2015) reported higher level of visfatin in subject with type 2 diabetes mellitus patients.

Takebayashi, *et al.*(2007) didn't find any correlation between visfatin and diabetes, and other study (Lim, *et al.*, 2008) proved that there is a positive correlation between the decrease of visfatin and type 1 diabetes. The mean values of visfatin were higher in diabetic group as compared to the obese and control group and there is negative correlations of visfatin with triglycerides, in diabetic patients. In the case of visfatin against HDLc, there were negative correlations in diabetic and obese patients and positive correlations in the control group (Gligor, *et al.*, 2012). Diabetic patients compared with healthy control group decreased serum adipo-nectin and increased serum visfatin levels may be useful in the elucidation of the connection between obesity - insulin resistance (Kara, *et al.*, 2014).

Plasma osteoprotegerin levels are elevated in newly diagnosed diabetic patients. (Xiang, *et al.*, 2006). Serum Osteoprotegerin (OPG) levels elevates significantly ($P<0.05$) in all patients compared with the control group (Hussain, *et al.*,2014) (Ahmed , *et al.*,2015).

Conclusions

1. There is highly decrease of resistin in diabetic patients.
2. There is highly decrease of visfatin in diabetic patients.
3. There is highly decrease of osteoprotegerin in diabetic patients.
4. Visfatin and osteoprotegerin increased in 11-15 years of duration of diagnosis.
5. There was no relation between resistin and duration of diagnosis.

Recommendations

1. Conduct more studies on resistin, visfatin and osteoprotegerin and their relationship to diabetes mellitus.
2. Conduct studies about genetic of diabetes mellitus included also insulin DNA sequences and insulin receptor
3. Conduct scientific research to discover drugs more effective than currently used in diabetes mellitus.

Acknowledgement

The authors are too grateful and thankful to diabetic patients and healthy control for their help to complete research of them, hoping a quick recovery for patients.

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