The association of serum resistin and other inflammatory markers with type 2 diabetic men

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Abstract

Objective: The evaluation of serum resistin level in type 2 diabetic patients as a good inflammatory marker and as a novel link between metabolic and inflammatory pathways in adipocytes and immune cells. Also we evaluate the serum levels of amyloid-A and C-reactive protein as low-grade inflammatory markers and as one of the elements in the maintenance of a proinflammatory state in diabetes

Material and methods: Eighty (80) patients aged from 45-60 years old were included in this study and were divided into 20 normal used as control and 60 with type 2 diabetes which are subdivided into 3 groups according to history and severity of type 2 diabetes. These 3 groups are type 2 diabetes without complication, with infection and with complication. Fasting glucose, resistin , amyloid-A and C-reactive protein were measured.

Results: We found that serum resistin , amyloid-A and C-reactive protein concentrations were increase significantly in type 2 diabetic patients compared to normal ones after adjustment for age ($P < 0.01$). **Conclusion:** Our data indicates that increase serum resistin, amyloid-A and C-reactive protein concentrations were associated with type 2 diabetes and considered as good inflammatory markers for incidence of this disease.

Keywords: Resistin; amyloid-A; C-reactive protein; Type 2 diabetes**.**

Abbreviations: CRP, C-reactive protein; DM, Diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; IL, interleukin; IR, insulin resistance; MetS, metabolic syndrome; SAA, Serum amyloid-A; T1D, Type 1 diabetes; T2D, Type 2 diabetes; TNF, tumor necrosis factor; WBC, white cell count; WHO, world health organization.

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Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders affecting almost 6% of the world's population. The prevalence of this chronic metabolic disease is on the increase[1]. On the basis of its etiology, DM can be classified into two major types. Type 1 diabetes (T1D), caused by immunological destruction of pancreatic islets, and is characterized by absolute insulin deficiency. Type 2 diabetes (T2D) begins in the middle age or earlier and is characterized by deficient insulin secretion and or insulin resistance[2].

Resistin, an adipocyte-derived hormone, which is associated with insulin resistance (IR) in vivo and in vitro, has been considered to link obesity with T2D. It serves as a signaling molecule between the energy storage organ, adipose tissue, and the principal insulin-responsive organs, liver, muscle and fat[3]. In addition to altering insulin sensitivity, resistin has been shown to cause disturbances in glucose metabolism[4].

Resistin was also shown to specifically accumulate in the inflamed joints at levels correlating with other markers of inflammation[5]. resistin levels correlate with markers of inflammation including C-reactive protein (CRP)[6] which is a common acute phase protein, i.e. a circulating protein manifesting with a very rapid and marked increment of its concentration in response to infections, traumatisms and, more in general, all kinds of tissue injuries[7]. Thus, resistin may be a novel link among metabolic signals and inflammation[8].

Serum amyloid-A (SAA) is a classic acute-phase protein predominantly produced by the liver in response to injury, infection, and inflammation[9]. It was proposed that SAA is a signal for redirecting high density lipoprotein (HDL) to sites of tissue destruction and cholesterol accumulation[10]. SAA could also modulate the activity of lecithin cholesterol acyltransferase (an enzyme responsible for cholesterol esterification), thereby affecting net cholesterol accumulation[11].

The purpose of this study is to evaluate the serum levels of resistin, amyloid-A and CRP as inflammatory markers in type 2 diabetic patients and to know the effect of this disease on its concentrations.

Material and Methods

Study population

Eighty patients their ages ranged from 45-60 years old were selected from the outpatient's department of Menoufiya University hospital and were classified into 2 major groups. First one was 20 healthy ones referred to as control; second group was 60 type 2 diabetic patients which subdivided into 3 minor groups according to history and severity of type 2 diabetes. These 3 minor groups are type 2 diabetes without complication, with infection and with complication. According to world health organization (WHO) criteria[12], diabetes was defined by either fasting plasma glucose levels ≥126 mg/dL, 2-h post-load glucose levels ≥200 mg/dL after a 75 g oral glucose tolerance test, or diabetes diagnosed by physicians. The principles laid down in the Declaration of Helsinki have been complied with. Also this study has been approved by the relevant local ethics committee, and all the subjects have given informed consent to participate in the research.

Anthropometric and Biochemical Measurements

After overnight fasting, venous blood samples were collected between 8 and 10 AM. Blood samples were collected in tubes containing EDTA for measuring fasting plasma glucose level; the remaining frozen serum samples were sent to laboratory and were stored in deep freezers at −70°C until used for measuring resistin , amyloid-A and CRP.

Fasting plasma glucose level was measured by the glucose oxidase method using a commercial kit (Spinreact, Santa Coloma, Sant Esteve De Bas, Spain). Serum resistin concentration was measured by enzyme-linked immunosorbent assay (ELISA) using a

commercial kit (BioVendor resistin ELISA ,Gmbh, Germany), sensitivity 0.033 ng/ml, intraassay CV 2.8% –3.4% and interassay CV 5.1% –6.9%. SAA concentration was measured by ELISA using a commercial kit (Invitrogen SAA ELISA, Camarillo, USA), sensitivity 4 ng/ml, intraassay CV 6.2% –7.4% and interassay CV 7.4% –7.8%. Serum CRP concentration was measured by Latex agglutination slide test for the qualitative and semiquantitative determination in human serum using a commercial kit (BIOTEC Laboratories Ltd, 32 Anson Road, UK), The sensitivity of CRP Latex assay was 6 (5-10) mg/l. Whereas the diagnostic sensitivity and diagnostic specificity were 95.6 % and 96.2 % respectively.

Statistical analysis

Data were expressed as the Mean ± SEM. Statistical evaluation was performed by one-way analysis of variance (ANOVA), followed by Tukey comparisons test (comparison of three or more groups) with the instate for windows statistical package for the social sciences (SPSS) software and a P value less than 0.01considered significant.

Results and Discussion

The present study highlighted two major observations: T2D in patients aged from 45-60 years old was associated with an overall increasing in resistin, amyloid-A and CRP levels; this increasing in resistin, amyloid A and CRP levels was associated with hyperglycemia and IR which are components of metabolic syndrome (MetS).

Many tissues are affected by proinflammatory cytokines and cause recognizable features of T2D[13]. The T2D is therefore associated with a general activation of the innate immune system, in which there is a chronic, cytokine-mediated state of low-grade inflammation, explaining most of the so-called the MetS[13,14]. Also, there is evidence from several studies that genetically determined alteration in components of innate immune system are also associated with several risk factors linked to the development of T2D and MetS[15].

Passing first through a stage of impaired glucose tolerance and or impaired fasting glucose concentration, frank T2D eventually develops in association with varying number of other clinical and biochemical features, which are themselves cardiovascular risk factors and are together called the MetS[16].

Inflammation is therefore may be a mechanism that links susceptibility factors for T2D, the most important of which is heritability[17], and known trigger factors, such overeating and

underactivity[18], increasing age[19]and psychological stress[20]. In addition, all these factors are linked to biochemical actions which caused by activated innate immunity that result in IR and impaired insulin secretion[21].

Resistin, secreted from adipocytes, that has been proposed to be the link between obesity, IR and T2D[22]. One of the areas of controversy on the potential role of resistin is the source of human resistin, the role of human resistin has been questioned because it is produced largely by macrophages in diabetes compared to normal subjects[23]. Thus, adipose tissue is an important inflammatory source in obesity and T2D, not only because of cytokines produced from the adipocyte itself (e.g tumor necrosis factor (TNF)-α, interleukin (IL)-6, resistin and reduced adiponectin)[24], but also because of infiltration by proinflammatory macrophages[25].

In our study we have shown that serum resistin level was mildly significant increased in group of patients with T2D without complication, moderately significant increased in group of patients with T2D with infection and markedly significant increased in group of patients with T2D with complication compared to normal subjects as shown in (Table 1) and (Fig. 1) and this increased levels of resistin appeared to be associated with significant increase in glucose levels in all 3 T2D groups. This is agreement with another study which suggested that resistin may be link between obesity, insulin resistance and diabetes[26].

Table (1) Glucose, Resistin, Amyloid-A and CRP levels in serum of studied patients and control

Biochemical parameters	Control	Diabetic without complication	Diabetic with infection	Diabetic with complication	P-value
Glucose (mg/dl)	96.15 ± 6.96	157.1 ± 9.62	321.95 ± 74.08	295.9 ± 51.48	P < 0.01
Resistin (ng/ml)	1.99 ± 0.33	3.64 ± 0.75	6.16 ± 0.72	10.16 ± 1.37	P < 0.01
Amyloid-A (µg/ml)	3.08 ± 0.95	347.89 ± 13.61	378.16 ± 1.61	369.67 ± 1.8	P < 0.01
CRP (mg/l)	5.8 ± 1.01	17.65 ± 3.22	89.6 ± 5.67	36.75 ± 3.73	P < 0.01

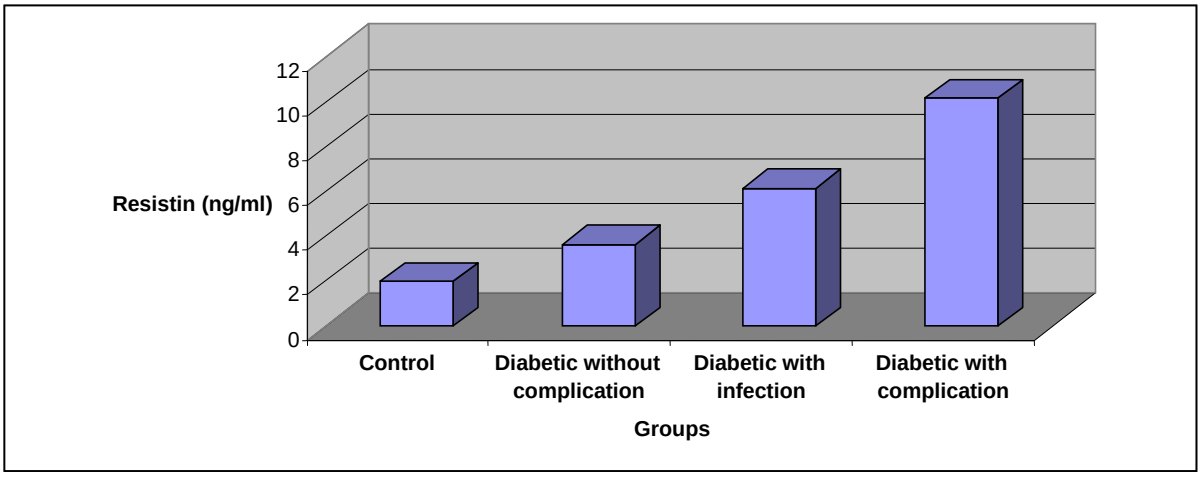

Figure 1 Resistin level in patients groups and control group.

Resistin level was mildly significant increased in group of patients with T2D without complication, moderately significant increased in group of patients with T2D with infection and markedly significant increased in group of patients with T2D with complication compared to normal subjects (P value < 0.01).

A study by Mojiminiyi and Abdella showed clearly that resistin increased with CRP with increasing degrees of low inflammation and white cell count (WBC), therefore resistin may be contribute to an increased risk of complications in patients with T2D[27].

Yaturu et al. reported that subjects with diabetes have decreased levels of adiponectin and elevated levels of CRP compared with control subjects[28]. It has been found that serum resistin is also associated with elevated CRP levels in T2D subjects, suggesting that this protein may be linked to subclinical inflammation[29].

Also Verma et al. suggested that plasma levels of resistin are associated with CRP and TNF-α suggested a role of resistin as a possible surrogate marker of inflammation[30]. our study were in agreement with those previous studies where we showed that CRP is markedly significant increased in patients with T2D especially in group of infection, also moderately significant increased in group of diabetes with complication and mildly significant increased in group of diabetes without complication compared to normal subjects as shown in (Table 1) and (Fig. 2) and this increased levels of CRP appeared to be associated with significant increase in resistin levels in all 3 T2D groups. So we can say that resistin appeared to be a

good marker of subclinical inflammation as it correlated strongly with other known proinflammatory cytokines, namely CRP.

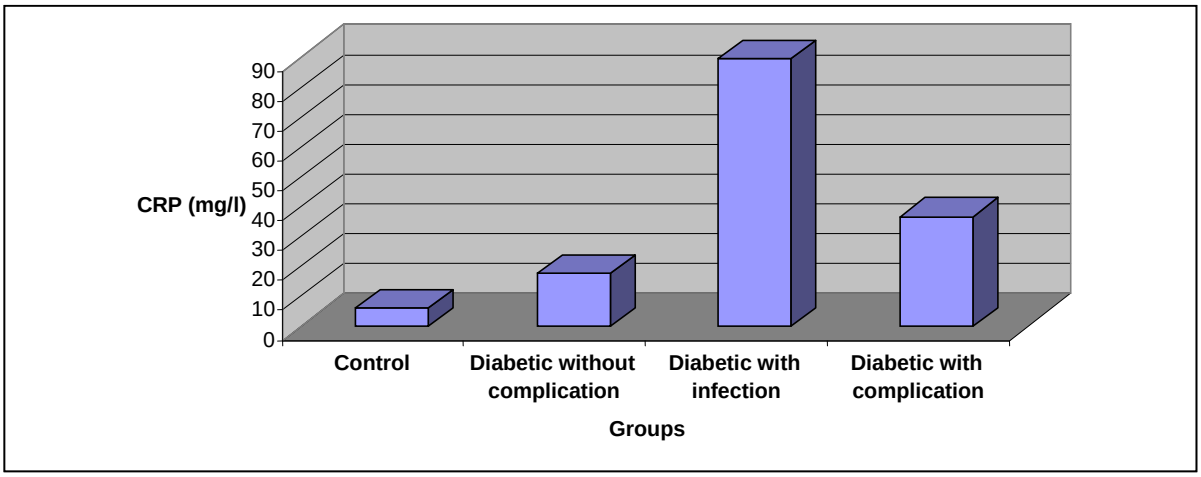

Figure 2 CRP level in patients groups and control group.

CRP level was mildly significant increased in group of patients with T2D without complication, moderately significant increased in group of patients with T2D with complication and markedly significant increase in group of patients with T2D with infection compared to normal subjects (P value < 0.01).

It has been suggested that CRP is not only a marker of inflammation but is also closely related to adiposity. This may raise doubt about whether CRP is a marker of inflammation or adiposity. The increased level of serum CRP in obese individuals is due to increased secretion of IL-6 and TNF-α in adipocytes, which regulate CRP production in hepatocytes and induce a chronic inflammatory state[31].

SAA protein is an acute-phase protein and a very sensitive marker reflecting an acute inflammatory state. Both SAA and CRP are synthesized in the liver upon stimulation by proinflammatory cytokines such as TNF-α and IL-6[32].

Increased SAA and CRP have both been detected in various clinical disorders associated with inflammation. It was noted that SAA might be complementary to CRP[33]. In addition, SAA has been suggested to be a better marker of disease activity with wider dynamic range and more rapid response, and represents a different type of acute-phase response than CRP[34].

Also Du et al. showed that short-duration patients with T2D had high level of circulating SAA and CRP and these levels of SAA and CRP were related to metabolic indexes[35].

In accordance with these earlier studies we also displayed that level of circulating SAA is markedly significant increased in all groups of patients with T2D whether they were without complication or with infection or with complication compared to normal subjects as shown in (Table 1) and (Fig. 3) and these increased levels of SAA appeared to be associated with significant increase in CRP levels in all 3 T2D groups.

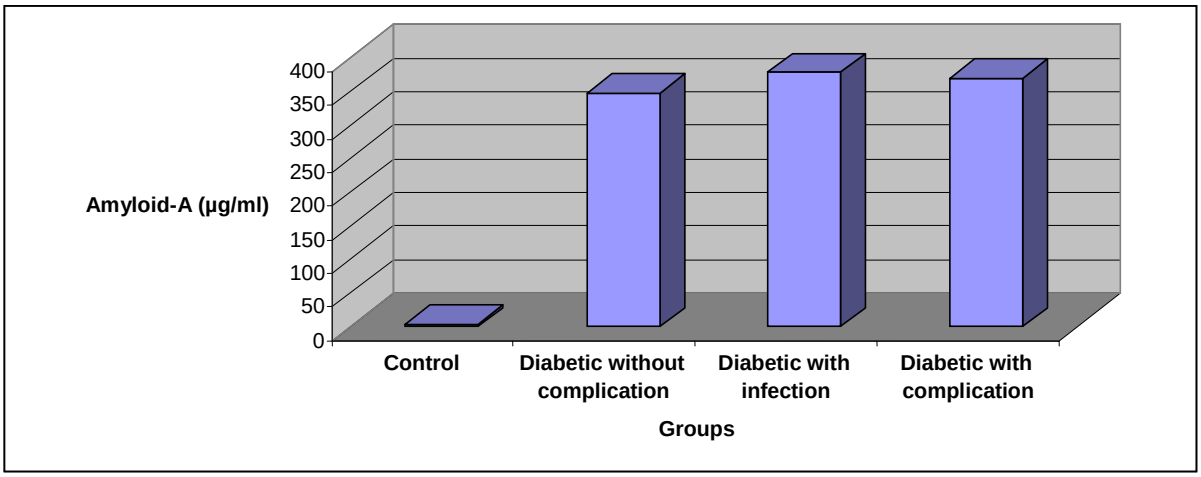

Figure 3 Amyloid-A level in patients groups and control group.

Amyloid-A level was markedly significant increased in all patients with T2D whether they were without complication or with infection or with complication compared to normal subjects (P value < 0.01).

Conclusions

In conclusion, we showed that serum resistin, amyloid-A and CRP concentrations were increased in type 2 diabetic patients and these increased levels could be considered as good biomarkers of the risk of T2D.

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