The Impact of Resistin and IL-6 On Type I Diabetes Mellitus (T1DM) and Its Duration in Children

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ABSTRACT:

BACKGROUND:

Type I diabetes mellitus is characterized autoimmune destruction of β- cells . IL-6 is a cytokine (one of a class of immune system regulators) it plays a role, in acute phase inflammatory response to cellular injury.

Human Resistin gene is expressed in pancreatic islets , pre- adipocytes and bone marrow and is of relevance for inflammation processes.

OBJECTIVE:

To investigate serum concentration of resistin and pro inflammatory IL-6 in T1DMchildren and to study the impact of the duration of disease on these parameter. In order to shed some light on the mechanism of initiation and propagation of β-cell damage in those patients.

SUBJECTS AND METHODS:

Sixty diabetes children (33 males and 27 females), aged (1.3-13) years (mean± SD) (5.6±2.8) years , consecutively admitted to the AL-Mansour Teaching Hospital for children , were enrolled in this study. All had T1DM . Duration of disease varied from (0.13- 84) months . Age matching group of thirty healthy volunteer children , (18 females ,12 males) was included as a control without any family history of diabetes. Resistin and IL-6 were measured (by enzyme linked immunosorbant assay , ELISA) .

RESULTS :

Fasting serum Resistin levels were lower in patients compared to controls although the correlation was not significant .However serum resistin levels were higher in females compared to males in both groups (patients and controls) with a significant correlation between the groups of the same gender .Duration of the disease had no impact on either gender.On the other hand IL-6 showed a significantly higher serum level in patients than control.

Un like Resistin , the duration of disease had a great impact on IL-6 serum levels as shown in text.

CONCLUSION :

- Resistin levels in patients with (T I DM) non significantly lower than in control individuals , that's meaning T I DM), as disease did effect the levels of serum Resistin sub clinically by the treatment with insulin leading to hyper insulinemia .Resistin antagonizes insulin action, leading to decrease in patients more than controls .
- Resistin levels tended to have higher in females than male ,however this trend did not reach statistical significant in total population due to sex hormone .
- The highly significant positive correlation between the levels of IL-6 and duration is due to persistente production and elevation for long time of patients with T1DM indicating of ongoing β- cell destruction .But Resistin was non dependent on the duration of the disease because human resistin gene is expressed in pancreatic islets cell.

KEYWORDS: resistin; IL-6; type 1 diabetes mellitus

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INTRODUCTION:

Diabetes can be classified into major classes:

1-Type 1diabetes[insulin dependent diabetes IDDM].

2- Type 2 diabetes (adult onset diabetes, non- insulin dependent diabetes NIDDM.

1-Type 1diabetes (juvenile onset diabetes): Is characterized by total exhaustion of insulin secretion [insulin dependent diabetes IDDM due to the fact that, the pancreas is unable to the synthesis of insulin. So glucose maintained from food can not be utilized

by cells.The principal treatment of type 1 diabetes, even from the earliest stages, is replacement of insulin. Without insulin, ketosis and diabetic ketoacidosis can develop and coma or death will result [1] .

Resistin is a 12.5 kD cysteine-rich peptide identical to FIZZ3 (found in inflammatory zone 3), and resistin- like molecules, such as RELM-α and RELM-β, are identical with FIZZ1 and FIZZ2, respectively. Patterns of expression of FIZZ proteins are very similar to those reported for resistin and RELMs [2] resistin/FIZZ3 and RELMs/FIZZ1 and FIZZ2 may be involved in the inflammatory processes associated with obesity[3].

Although Resistin was first discovered in 2001 and was originally found to be produced and released from adipose tissue to serve endocrine functions likely involved in insulin resistance[2], more and more evidence indicated that it may also be involved in inflammatory process. Some pro-inflammatory agents, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and lipopolysaccharide (LPS), can regulate Resistin gene expression. Resistin mRNA was strongly increased by TNF-α in human peripheral blood mononuclear cells (PBMC) [4]. IL-6 also increased resistin expression in peripheral blood mononuclear cells[4].

Recent studies have shown the regulation of pro inflammatory cytokine expression by resistin[5]. Resistin strongly unregulate IL-6 and TNF-α in human peripheral blood mononuclear cells [5].Other evidence linking resistin to inflammation is that plasma resistin levels were found associated with many inflammatory markers in some pathophysiological conditions[6].

THE AIM OF THE STUDY :

To investigate the impact of the duration of IDDM in children on serum levels of pro inflammatory markers takes is Resistin and IL-6 as examples . In order to understand of the mechanism behind the intiation and propagation of β-cell damage in those patients.

PATIENTS AND METHODS:

Sixty children (33 males and 27females), aged range (1.3-13) years (mean± SD) (5.6±2.8) years were enrolled in this study. All were consecutively admitted to the pediatric clinical of AL-Mansour Hospital through the period from January to August 2008 .All had Type 1 Diabetes Mellitus T1DM as diagnosed by a physician and used short-and intermediate acting subcutaneous insulin injections (two daily injections only) with no other medication .Duration of disease varied from (0.13- 84) months .Disease duration was defined in this study as the day of initial diagnosis of diabetes to the day of blood collection. Only subjects without hypertension , microalbuminuria , retinopathy , neuropathy or signs of ketoacidosis were recruited .Patients with disorders affecting metabolic parameters such as hypercortisolism, thyroid disease ,abnormalities in sex hormone regulation and patients with positive C-reactive protein were excluded. Informed consent was obtained from the parents of the children and the study was approved by the Departmental Committee. All patients had history of stress prior to presentation. Thirty non diabetic healthy children , similar in age and sex were selected as control subjects (12females and18males).Eight milliliters (ml) of venous blood sample were collected; using plastic disposable syringes then were separated by centrifugation at (300 rpm) for 15 min. The sera separated were stored frozen at (-20 ^{O}C) until assayed. Serum Resistin and IL-6 were measured by enzyme linked immunosorbent assay. **(**ELISA, Sandwich assay). The quantitative determination of glucose was done by the enzymatic colorimetric method immediately after separation.

RESULTS:

Table 1 shows the value of serum Resistin and IL-6 in both groups (patients and control) and in both genders (males and females).as shown in the table serum Resistin levels had no significant difference in both groups. On other hand there was a significant difference in the levels of serum Resistin in both genders when compared in each group as shown in table (1) an Fig (1).

The serum IL-6 levels had significant difference in both groups. On other hand there was a significant difference in the levels of serum IL-6 in both genders when compared in each group but no significant difference in the levels of serum IL-6 in both genders when compared in the same group as shown in table (1) an Fig (2).

Table 1 :The levels of Resistin (ng/ml/) and IL-6(pg/ml) in non diabetic controls and in patients with type I diabetes

Parameter	Type I Diabetic			Controls		
	Male	Female	Total	Male	Female	Total
Resistin(ng/ml) (mean± SD)	2.9± 1.66 N=30	3.8±2.0 N=30	3.5±1.83	3.31±2.23* N=15	4.25±1.8 * N=15	3. 8±2.02
IL-6(mean± SD)	33.1±9.02*	36.8±9.81*	34.9±9.5*	25.98±1.64	26.41±1.48	26.19±1.6

* P< 0.05 comparison between different group Significant

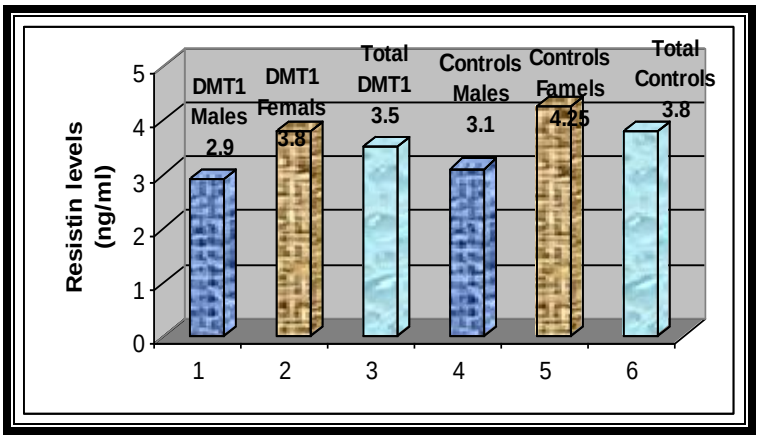

Fig. 1: The mean levels of Resistin (ng/ml) in male , females and total in patients with diabetes type 1 and healthy control .

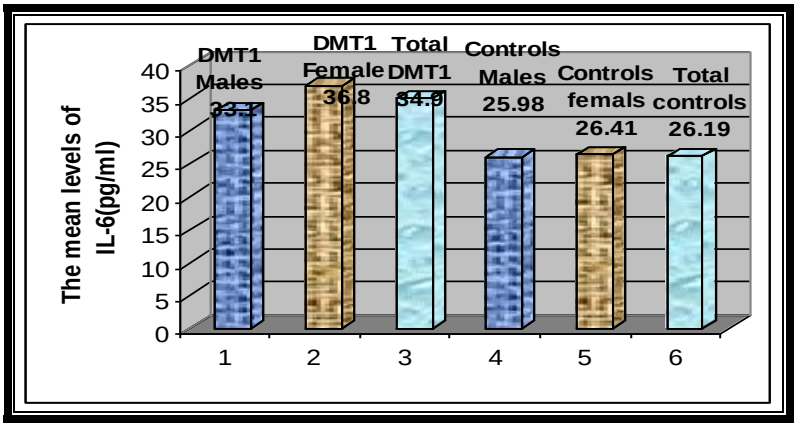

Fig. 2: The mean levels of IL-6 (pg/ml) in male , females and total in patients with diabetes type 1 and healthy control

Although IL-6 serum levels were higher in patients group compared to the control. The value were highly dependent on the duration of the disease as shown in Table (2), (Fig 3) . The value of serum Resistin levels was non dependent on the duration of the disease as shown in Table (2).

Table 2: The (mean± SD) levels of Resistin and IL-6 (pg/ml) in patients with diabetes mellitus type I and controls .

	Newly diagnosis IDDM ND -IDDM	Long standing IDDM LS-IDDM	Control	P Value
n	29	31	30	
Resistin(ng/ml)	3.1±1.56	3.8±2.1	3. 8±2.02	NS
IL-6 pg/ml (mean± SD)	26.28±2.22	42.8±7.88	22.9±3.9	*P<0.05 **P<0.05 ***P<0.05
Duration range (months)	0.13-6	12-84	--------	

* Correlation between ND-IDDM and control. NS= Non significant
** Correlation between LS-IDDM and control
***Correlation between ND-IDDM and LS-IDDM

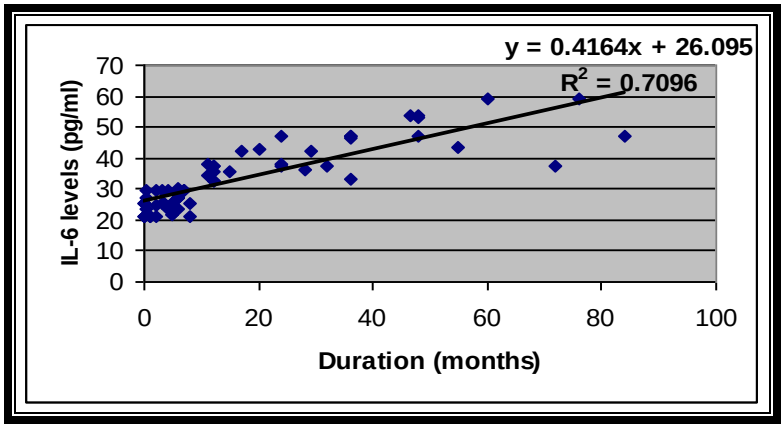

Fig. 3: The correlation between the levels of IL-6 (pg/ml)and duration (months) r= 0.8.

There is positive correlation between IL-6 (pg/ml)levels and Resistin (ng/ml) as shown in Fig (3),(r= 0.2), p<0.05.

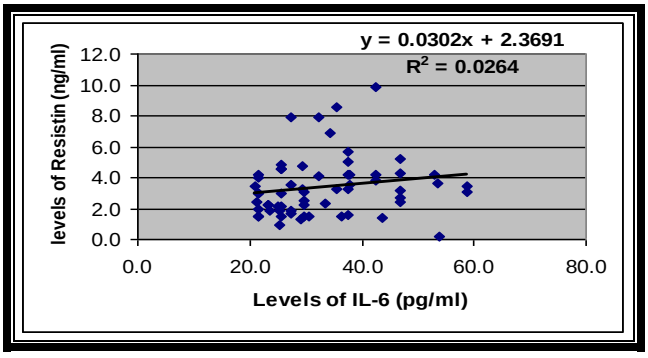

Fig. 4: The correlation between the levels of IL-6 (pg/ml)and Resistin levels (ng/ml) r= 0.2 .

DISCUSSION:

In patients with type 1 DM, the beta cells are attacked by the immune system (specifically by macrophages and T lymphocytes) and killed, so insulin can no longer be synthesized [7].

Patients with type 1 have non significant levels of fasting serum Resistin than healthy controls . as shown in table (1), the underlying pathogenesis of type1 diabetes dose not seem to be a critical issue.

Resistin reduces glucose up take by adipocytes and skeletal muscle and reduces insulin tolerance. In patients with DMT1was hyperinsulinemia due to insulin injection and Resistin antagonizes insulin action lead to decrease serum Resistin levels in compared to controls . The non –significant lowering of fasting serum Resistin of the patients than controls , that's meaning no effect of disease per sc could be detected.

Based on an overall interpretation of the results of this study , there is a relationship between diabetic status and adipocytokines . The effect that insulin treatment has on the various tissue (with the exception of its physiological effects) must also be taken into consideration due to reduced insulin activity in the liver , as opposed to chronic hyperinsulinaemia[8].

Our finding is in agreement with Fehmann HC,et al, [9] who study type 1 diabetes is an autoimmune disease. Serum Rresistin levels were firstly found not to be different between patients with type 1 diabetes and healthy controls . However, a recent study showed that healthy people had significantly higher plasma Resistin levels than patients with type1diabetes Schaffler A,et al. [10]

Two studies on plasma Resistin concentrations in T1DM furnished contrasting results Fehmann HC,etal and Shalev A, et al[11)][9]. An increase in serum resistin values was observed in the non-diabetic children involved in this study compared with T1DM no influence of sex. .

Patients with type I diabetes had non significantly ($p>0.05$) lower resistin levels than non diabetic controls but significant difference when compared the gender in patients diabetes and controls . Over all, female tended to have higher resistin levels than males . This phenomenon was evident in both genders and in the total population (Table1) Fig (1) .

Higher Rresistin levels has been found in girls compared with boys of corresponding age in children. This is in accordance with some studies carried out M. Gerber, et al [12]and Schaffler A, et al [10] who found female tended to have higher Resistin than males but this trend did not statistical significance in over all population , controls or patients type1 diabetes . Other investigators did not detect any differences between genders using various assays Youn BS,et al[13].

Because Resistin levels of male subjects are in absolute values only marginally lower than those of females, these differences may be related to sex hormone (human Resistin is barely detectable in adipose tissue and which was increase in females than male) Besides, the gender dependency may be more pronounced at a younger age [12] .

Chemokines play a central roles in inflammatory process by regulating leukocyte migration into sites of tissue damage [14]. Cytokines have been proposed as inducers of β- cells damage in human IDDM via the migration of NO[15]. The pro inflammatory cytokines IL-6 and TNF-& are common to both T_H subsets in human [14] TNF -& and IL-6 mediated damage to micro- and macrovasicular tissue , altered insulin secretion through direct or through stimulation of free fatty acid production , and altered glucose homeostasis are suggested [16] .IL-6 and TNF-and are adipocyte -secreted factors [17]. The pro inflammatory cytokine production is in diabetes and in case of metabolism have the potential to influence macrophage cytokine release inducing up regulation of pro inflammatory Cytokines [18].

IL-6 might play a significant role in IDDM etiopathogenesis [19] .Diabetic patients have elevated blood levels of IL6, which is known to increase the inflammation and development of vascular disease and atherosclerosis [20]. IL-6 levels were found to be increased statically significantly in any group of children with IDDM especially in newly diagnosed ($P<0.05$) cases when compared with healthy controls. Even a statistically significant difference was revealed between IL-6 value related to newly diagnosed cases and those found during long standing period ($P<0.05$) table (2).

In our study IL-6 levels did not reach to those of healthy children even through they raised from their lowest values in newly diagnosed case, and increased further in IDDM patients monitored for long term .In this study serum pro inflammatory cytokine concentration IL-6 indicated a state of chronic inflammation in all patients with IDDM , levels of IL-6 in all DM groups were detected to be

significantly different from those of healthy controls. Our data shows decreased serum IL-6 levels in newly diagnosed IDDM patients in comparison with long standing case supported an activation of systemic inflammatory process during early phase of IDDM which may be indicative of an ongoing β-cells distraction . Persistence of significant difference

between the cases with IDDM monitored for long time and controls in terms of IL-6 supports continuous activation during the late stage of diabetes.

Our study is in agreement with Yasar Dogan, et al [21] that IL-6 levels were found to be increased statically significant in any group of children with IDDM especially in newly diagnosed cases when compared with healthy controls. However in some studies IL-6 levels were found to be higher in newly diagnosed case when compared with those monitored for long time Erbagci AB,et al., [14] .The reports of IL-6 abnormal production in patients with IDDM are rare [22] .

We found highly positive correlation between the levels of IL-6 (pg/ml) and duration (months) r= 0.8, P<0.05. as shown in Fig (3).

Our study is in agreement with Yasar Dogan, et al [22] that IL-6 levels were found in positive correlation with duration and no dependency to gender .

IL-6 persistence production and increased for long time of patients with DMT1 indicative of ongoing β-cell destruction .The value of serum Resistin levels was non dependent on the duration of the disease as shown in Table (2).Our finding agrees with A. Schaffler et al.,[10] who found that disease duration has no affect on serum Resistin levels. This might due to the fact that human Resistin gene expressed in pancreatic islets [10] and there is no time dependency.

Resistin, which has recently been proposed to play a role in obesity-mediated insulin resistance[2], has a structure similar to that of proteins that are involved in inflammatory processes [23]. More specifically, resistin is identical to FIZZ3 (found in inflammatory zone 3), and resistin- like molecules, such as RELM-α and RELM-β, are identical with FIZZ1 and FIZZ2, respectively. Patterns of expression of FIZZ proteins are very similar to those reported for resistin and RELMs [2 . In addition, the pattern of expression and physiological functions proposed for these proteins resemble those of other well-known proinflammatory cytokines, such as interleukin-6 and TNF-_, both of which are involved in cardiovascular obesity-related outcomes [24] . These findings suggest that resistin/FIZZ3 and RELMs/FIZZ1 and FIZZ2 may be involved in the inflammatory processes associated with obesity[25], but the role of resistin in vascular reactivity or potential associations with inflammatory markers have not been previously studied. Larger studies are needed to confirm and extend these finding in healthy and diabetic subjects.

We found positive correlation between the levels of IL-6 and Resistin levels as shown in Fig (4).

Recent studies have shown the regulation of pro inflammatory cytokine expression by resistin. Resistin strongly unregulated IL-6 and TNF-α in human peripheral blood mononuclear cells[26]. Addition of recombinant human resistin protein to macrophages from both mouse and human resulted in enhanced secretion of pro-inflammatory cytokines, TNF-α and IL-12 [25].

Stejskal D, et al.,][27] was showed that people with severe inflammations, a significant positive correlation between resistin and inflammatory markers, this agreement with our results.

CONCLUSION:

1-The study showed an in dependent association between Resistin and type I diabetes . that's meaning IDDM, as disease did effect on levels of serum Resistin sub clinically by the treatment with insulin lead to hyper insulinemia .Resistin antagonizes insulin action lead to decrease in patients than controls .

2-Resistin levels tended to be higher in females than male ,however this trend did not reach statistical significant in total population due to sex hormone.

3- The highly significant positive correlation between the levels of IL-6 and duration is due to persistence production and elevation for long time of patients with IDDM indicative of ongoing β- cell destruction .But Resistin was non dependent on the duration of the disease because human resistin gene is expressed in pancreatic islets and there is no time depency.

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