Original paper

Difference between Pro- & Anti-Inflammatory Cytokines in Latent Autoimmune Diabetes of Adult (LADA) and Diabetes Mellitus Type2 (D.M.2)

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Abstract

Background: Immunological profile of LADA is not so clear, so detection of cytokines is relevant to determine the extent and direction of immune responses.
Objective: the aim of the study is to identify role of pro-& anti-inflammatory cytokines in diabetes progression and their relation with diabetes complications.
Methodology: level of the pro-inflammatory cytokines IL-6 and IFN-γ and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1RA) were estimated in 90 subjects (34 known LADA cases, 36 D.M.2 cases & 20 healthy control participants), participating patients were recruited from Diabetes outpatient clinic in AL-Hussein Teaching Hospital in Karbala from June, 2013 through January, 2014. A clinical questioner containing personal data, family history, type of diabetes, hemoglobin A1C(HA1C), body mass index (BMI), diabetes duration& complications of diabetes was obtained from all patients. Statistical analysis done by using the statistical package for social sciences (SPSS) software for windows, data of all participants were entered and analyzed with appropriate statistical tests.
Results: levels of IL-6 & IL-1RA were significantly higher in D.M.2 and LADA cases than controls & both are associated with complications in D.M.2 patients, in addition to positive significant correlation between them. Regarding IFN-γ, it was significantly higher in LADA group than D.M.2 group.
Discussion: Levels of IL-6 & IL-1RA are increased in complicated cases for both LADA & D.M.2 groups & IFN-γ is more in LADA group.
Conclusion: up regulation of both pro- and anti-inflammatory mediators to ameliorate inflammation in pathogenesis of diabetes.
Recommendation: Further studies on IL-1RA in diabetes are recommended to see the useful effects of IL-1RA in preserving β-cell function and its therapeutic role in both autoimmune & type 2 D.M.
Key word: IL-6, IFN-γ, IL-1RA, LADA, Body mass index, diabetes mellitus.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism occurred due to defects in insulin secretion, action, or both [(1)]. Diabetes mellitus can be classified into four types according to etiology; D.M.1, D.M.2, gestational diabetes and other specific types of diabetes. The majority of cases of diabetes fall into type 1 diabetes or type 2 diabetes [(1)]. Type 2 diabetes is characterized by impaired β cell function and may be accompanied with changes of the immune system [(2)]. Latent autoimmune diabetes in adults (LADA) has some clinical features of type 2 diabetes & shows immunological abnormalities similar to those in type 1 diabetes, such as glutamic acid decarboxylase antibody (GADA) [(3)]. So far it is not understood

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why disease progression in LADA is slower than in type 1 diabetes despite the immunological similarities. Insulin secretion was reported to be intermediate in LADA compared with type 1 and type 2 diabetes, whereas metabolic syndrome was similar in type 1 diabetes and LADA [4].

The microenvironment has very important role for directing the T-cell response towards type 1 or type 2 cytokine secretions. Previous studies found an increment of systemic pro- and anti-inflammatory cytokine levels in patients at risk for diabetes and with classical type 2 diabetes [5; 6]. Patients with elevated BMI with or without type 2 diabetes also have high levels of systemic cytokines [5; 7]. Especially, IL-6 and TNF-α are produced in high concentrations in adipose tissue and are thought to be attributed to both development of type 2 diabetes and insulin resistance [8]. Serum concentrations of IL-1RA (anti-inflammatory cytokine) are also produced in adipose tissue and are associated with obesity and disease progression of type 2 diabetes [9].

During pathogenesis of type 1 diabetes invading immune cells secret cytokines such as IL-1β, TNF-α and IFN-γ [10; 11]. After development of type 1 diabetes, T cell reactivity and systemic cytokines secretion such as IL-1RA are associated with endogenous insulin secretion and have been shown to relate to disease progression [12; 13]. Treatment with IL-1RA in type 2 diabetes patients has shown to decrease HA1c and to improve endogenous insulin secretion [14]. Besides the role in the evolution of autoimmune diabetes mellitus, IL-1 and IL-1Ra appear to play an important role in the pathogenesis of diabetes mellitus type2, metabolic stress caused by repetitive glucose excursions, dyslipidemia and increased levels of adipokines can induce an inflammatory response characterized by local cytokine secretion, islet immune cell infiltration and β-cell apoptotic death which can be important in the pathogenesis of diabetes mellitus type 2 [15].

So the current study aimed to clarify the role of these pro- and anti-inflammatory cytokines in the pathogenesis and progression of diabetes.

Patients and Method

All patients who entered this cross-sectional study were selected randomly from Diabetes outpatient clinic in AL-Hussein Teaching Hospital in period from June 2013 through January 2014. The study population consisted of 90 individuals; the patients group aged from 30 to 73 years and with duration of disease between 1 month -25 years, 36 of whom have been diagnosed clinically as type 2 D.M., 34 patients was known LADA cases (having Glutamic acid decarboxylase antibody positive result) & 20 healthy control subjects. Serum samples were collected and stored at freezer (all samples allowed to be thawed only once) for cytokine analysis. Circulating cytokine concentrations of IL-6, IL-1RA & IFN-γ were measured by ELISA technique using commercially available kits(IL-6 Human ELISA Kit (abcam, Englad US), Human IL-1RA platinum ELISA kits (eBioscience, USA), Human IFN gamma ELISA Ready-Set-Go (eBioscience, USA)). Statistical method: by using the statistical package for social sciences (SPSS) software for windows, version 20, IBM, US, 2010, data of all participants were entered and analyzed with appropriate statistical tests. Descriptive statistics were presented as mean and standard deviation (SD) for the continuous variables and as frequencies and proportion for the categorical variables (No. and %). Analysis of variances (ANOVA) test was used to compare means of variables for three groups, and student's ***t*** test was used to compare means for two groups. Bivariate correlation was used to estimate the correlation between marker and other variable. Linear

regression (curve estimation) was used to assess the significance and the direction of the correlation between IL-6 and IL-1RA, Pearson correlation coefficient (R) value represented the strength of the correlation and the sign of R represented the direction of the correlation as followed: R value of < 0.4 indicated a weak correlation, 0.4 – 0.7 indicated moderate correlation, and R > 0.7 indicated a strong correlation. The minus sign indicated an inverse (negative) correlation and the no-sign value indicated positive (direct) correlation. Level of significance (P-value) was set at ≤ 0.05 as a cutoff point for significant difference or correlation.

Results

Table (1) shows that there is a statically significant differences between D.M.2 vs. controls, and LADA vs. controls (P<0.05), while no significant difference had been found in between LADA and D.M.2 groups, P>0.05. Regarding the mean IFN-γ, it was significantly higher in LADA group than D.M.2 group (P=0.032), while the difference in mean IFN-γ was statistically not significant neither between LADA and controls nor D.M.2 vs. controls, in both comparison, P>0.05. The mean IL-1RA was significantly higher in D.M.2 and LADA cases than controls (P<0.05), while the difference was statistically not significant when compare D.M.2 vs. LADA cases, (P>0.05).

Table 1. Comparison of mean values of cytokines markers of D.M.2, LADA & control groups

Marker	DM2 (No.=36)	LADA (No.=34)	Control (No.=20)	Compared groups	*P-value*
IL-6 (pg/ml)	11.6 ± 3.1	12.4 ± 1.7	0.95 ± 0.14	DM2 vs. LADA	0.97 *NS*
				DM2 vs. control	**0.014 *sig***
				LADA vs. controls	**0.009 *sig***
IFN-γ (pg/ml)	40.3 ± 4.5	87.5 ± 20.3	44.28 ± 6.2	DM2 vs. LADA	**0.032 *sig***
				DM2 vs. control	0.98 *NS*
				LADA vs. controls	0.12 *NS*
IL-1RA (pg/ml)	1223.6 ±102.8	1226.8±37.2	701.4 ± 41.4	DM2 vs. LADA	0.96 *NS*
				DM2 vs. control	**0.011 *sig***
				LADA vs. controls	**0.010 *sig***

* NS: not significant, sig : significant.*IFN- γ = interferon gamma

In table (2) a positive significant correlation was found between IFN-γ, IL-1RA & HA1C. Other markers showed mild to moderate correlation with variables (according to the value of R), although this correlation was statistically not significant.
In type 2 D.M. markers are higher in obese patients & overweight categories although result still statistically non-significant, see table (3).
Table (4) shows that IL-6 & IL-1RA are associated with increased complications in D.M.2 group.
There was a highly significant positive correlation between IL-6 and IL-1RA, (R= 0.35, P=0.001), see figure (1).

Table 2. Correlation between circulating cytokine concentrations and variables for all patients groups combined

Variable	IL-6	p-value	IL-1RA	p-value	IFN-γ	p-value
HA1C	0.11	0.4	0.31	**0.01***	0.31	**0.01***
BMI	0.15	0.144	0.244	0.20	- 0.070	0.52
Duration of diabetes	0.023	0.85	- 0.19	0.11	- 0.064	0.60
Age	0.082	0.44	0.005	0.96	- 0.045	0.67
Gender	- 0.06	0.55	0.08	0.45	- 0.18	0.095

***** *Correlation is significant at P<0.05, Hyphen-minus sign indicated inverse (negative) correlation.*

Table 3. Comparison of mean values of markers according to BMI categories of DM2 & LADA cases. (Values represented the mean values of the markers)

BMI / DM2	Normal (18 - 24.9)		Overweight (25 - 29.9)		Obese (>=30)		p-value
	Mean	SD	**Mean**	SD	**Mean**	SD	
IL-6(pg/ml)	**3.7**	.7	**15.5**	4.9	**9.9**	4.4	0.52
INF-γ(pg/ml)	**33.0**	7.5	**44.5**	7.0	**33.9**	3.4	0.51
IL-1RA(pg/ml)	**941.3**	137.3	**1234.0**	149.0	**1406.4**	176.1	0.98
BMI / LADA							
IL-6(pg/ml)	13.5	3.4	13.2	2.2	9.2	4.8	0.36
INF-γ(pg/ml)	115.5	56.8	76.4	18.7	65.6	7.3	0.65
IL-1RA(pg/ml)	921.5	144.2	1537.0	221.0	849.4	146.6	0.062

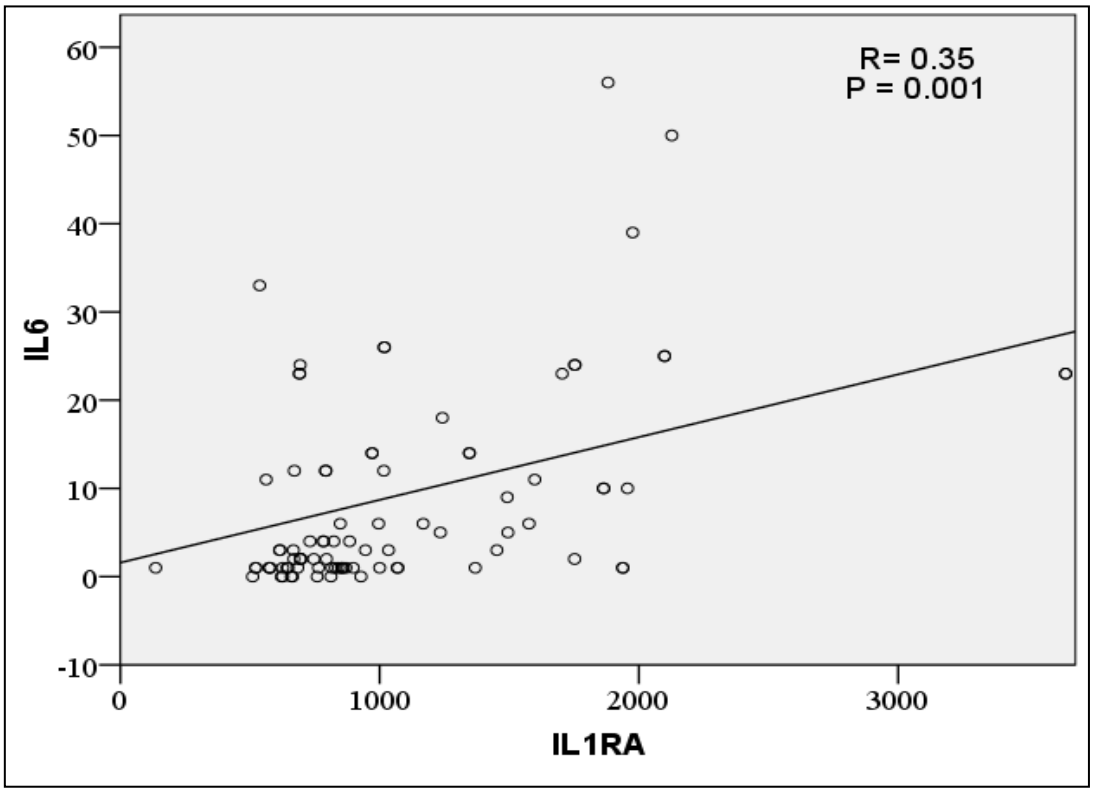

Figure 1. Correlation between IL-1RA and IL-6 (P=0.034)

Table 4. Comparison of mean values of markers according to complications in DM2 & LADA cases. (Values represented the mean values of the markers)

Marker for D.M.2	Complications					P-value
	Retinopathy	diabetic foot	IHD	CVA	Nephropathy	
IL-6(pg/ml)	4.5	20.5	6.0	56.0	8.0	**<0.001***
INF-γ(pg/ml)	34.6	34.0	49.5	52.0	18.0	0.79
IL-s1RA(pg/ml)	1150.0	1473.0	888.0	1881.0	3740.0	**0.001***
Markers for LADA						
IL-6(pg/ml)	16.7	6.0	None	None	None	p>0.05
IFN-γ(pg/ml)	54.7	37.0				
IL-1RA (pg/ml)	1409.3	1168.0				

* P. value is significant at <0.05 level.

Discussion

In the present study revealed significant high levels of IL-6 in both LADA & D.M.2 groups compared to control group this is in agreement with Pham MN, *et al.* study 2012 [16]& Xiang Y, *et al.* 2011 [17]**.**
A study done in Germany showed that the level of pro-inflammatory (IFN-γ) was more in LADA group compared to D.M.2 & control groups & this result fits ours [18]**.**
It has been shown that during pathogenesis of autoimmune diabetes the recruiting immune cells produce cytokines such as IFN-γ, which are known to be cytotoxic to beta cells [19; 10; 11]**.** This explains increase level of IFN-γ in LADA patients**.**
Regarding correlation of cytokines with HA1C, positive significant correlation was found with IFN-γ and IL-1RA. This goes with study done in Mexico City which revealed positive association between HA1C & IFN-γ [20]**,** also similar to report of Raz *et al.* which shows positive correlation between HA1C & IL-1RA [21].
For the rest variables correlation (BMI, age, gender, duration) no statistical difference was found, but Cytokines are higher in overweight &obese D.M.2 cases. **A** previous study illustrated that the highest median systemic cytokine concentrations of IL-1RA& IL-6 were in individuals with obesity, followed by overweight patients and with the lowest values in patients with normal weight and this result matches our current study [16]. These results point to a positive influence of obesity on the production of systemic cytokines regardless type of diabetes and it is an additional risk factor for impairment of disease progression in autoimmune diabetes as well as type 2 diabetes. Several studies have revealed an association between overproduction of pro- and anti-inflammatory cytokines and weight, obesity& adipose tissue [22]**.**
Current study shows significant positive correlation between IL-1RA & IL-6.This result is in agreement with report of Pham *et al.* [16]. Explanation for this result may be due to reflect of a counter-regulatory attempt between pro- and anti-inflammatory cytokines to ameliorate inflammation in patients with diabetes, associated with up regulation of immune responses.
Levels of IL-6 & IL-1RA are significantly associated with different complications in type 2 D.M. This result fits study of Jong-Han *et al.* which revealed increase levels of IL-6 in D.M.2 patients with complications [23] & goes with other study which showed association between increments of IL-1RA in D.M.2 & complications [24].

Conclusion

Increased BMI affects systemic cytokine concentrations in patients with type 2 diabetes, IFN-γ is associated with autoimmunity in diabetes and Up regulation of both pro- and anti-inflammatory mediators in pathogenesis of diabetes.

Recommendation

1. A longitudinal prospective study suggested to be performed on diabetic patients to investigate the effects of pro- & anti-inflammatory cytokines on development & complications of diabetes.
2. Further studies on IL-1RA in diabetes are recommended to see the useful effects of IL-1RA in preserving β-cell function and its therapeutic role in both autoimmune & type 2 D.M.

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