Autoantibodies and Cytokines Levels in Type 1 Diabetic Patients

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ABSTRACT:

BACKGROUND:

Type 1 diabetes is characterized by a complete or near-complete insulin deficiency caused by an immune-mediated selective destruction of the insulin-producing β-cells in the Islets of Langerhans. Inflammatory mechanisms play a key role in the pathogenesis of type 1 diabetes. Many findings suggest that the Islet autoantibody status in type 1 diabetes is linked to disease activity.

OBJECTIVE:

To investigate the hypothesis that the systemic immunoregulatory balance, as defined by levels of circulating cytokines, is associated with Islet autoantibody status.

METHODS:

Cytokines (IL-2, IL-4, IL-5,IL-10, TNF-β and INF-γ) and Islet autoantibodies (ICA, GADA, IA-2) were measured in 56 patients with insulin dependent diabetes mellitus (IDDM) and 20 healthy control patients.

RESULTS:

The three proinflammatory cytokines measured [interleukin-2 (IL-2) , interferon gamma (IFN-γ) and tumor necrosis factor-β (TNF-β)], both TNF-β (50.0 ±5.9) (63.4± 5.4) and INF-γ (13.8 ± 10.9) (13.7 ± 5.5) showed a significant increase ($P < 0.05$) with Islet autoantibody positivity, while the other three cytokines,(IL-4,IL-5 and IL-10), only IL-4 showed a positive increase (54.4 ± 1.4) with Islet autoantibody positivity although it is non- significant association.

CONCLUSION:

The study reveals the possibility of the of Islet autoantibodies in the domination of proinflammatory cytokines over the immunoregulatory cytokines.

KEY WORDS: type 1 diabetes, Islet autoantibody, proinflammatory cytokines, immunoregulatory cytokines.

INTRODUCTION:

Type1 (insulin-dependent) diabetes mellitus (IDDM) is an autoimmune disease characterized by insulin insufficiency that results from a progressive immunological destruction of insulin-secreting pancreatic Islet ß-cells by autoreactive leukocytes and their mediators [(1)]. Type 1 diabetes is associated with the appearance of humoral and cellular Islet autoimmunity, and a defective immunoregulation appears to be involved [(2,3)].

Once β- cells are damaged, hypothetically, sequestered antigens are then released to which the immune system responds (e.g., GAD and IA-2) that may not be β- cells specific. Thus, over time, more islet autoantibodies appear and epitope spreading occurs [(4)]. Prominent Islet autoantibody types include: glutamic acid decarboxylase antibodies (GADA), insulinoma-associated antigen 2 antibodies (IA-2A), and Islet cell antibodies (ICA), a mixture of various autoantibodies binding to Islet cell cytoplasm constituents in cryostat sections of human pancreas [(5)].

An important finding was that the highest risk is associated with the presence of two or more different Islet autoantibody species and this observation suggests that the Islet autoantibody status may distinguish mild from strong disease activity [(6)]. After clinical onset of the disease, different characteristics have been noted for patients who were either positive or negative for Islet autoantibody [(7,8)]. In addition, high and multiple Islet autoantibody titers may be an early sign of a rapid disease progression in terms of loss of residual B-cell function during the first years of disease [(9,10)]. It was assumed that presence of these autoantibodies may disturb the immunoregulation balanced which define by levels of circulating cytokines and chemokines [(11)].

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The cytokines interleukin (IL)-1, tumor necrosis factor, interferon γ, IL-6, IL-10, IL-15, IL-17, and monocyte chemoattractant protein-1 have been implicated in the development of microvascular and macrovascular complications in both IDDM and type 2 diabetes[12,13]. While several studies have demonstrated strong correlations between proinflammatory cytokines and endothelial damage, it remains to be demonstrated whether a cause-and-effect relationship exists between proinflammatory cytokines and the pathogenesis of diabetes complications. Whereas some investigators hypothesize that cytokine alterations play an important role in disease pathogenesis, others contend that cytokine changes are a protective physiologic response to hyperglycemia-induced stress [14,15,16]. Since cytokines have been linked to the development of autoimmunity, including that to the beta cell, it is important to consider that an imbalance between proinflammatory and anti-inflammatory cytokine activities may favor both the induction of autoimmunity and the chronic inflammation that leads to complications[17,18].

The aim of this study was to study the role of autoantibodies in type 1 diabetes in disturbance of systemic immunoregularty balanced by measuring serum concentration of the mediators: IL-2, IL-4, IL-6, IL-, TNF-β and INF-γ.

MATERIALS AND METHODS:

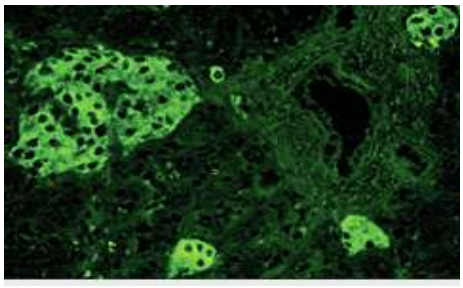
PATIENTS:

Fifty six IDDM patients (29 female, 27 male), mean age 16.1±2.5 ranging between 2-29 years, and 20 normal healthy controls, mean age 19.7±3.1, ranging from 5 to 35 years, participated in the study. The patients were visiting Al-Kindy Diabetes Center regularly. All patients were treated with daily regular doses of insulin. At the time of enrollment, none of the diabetic patients or controls suffered from any other significant acute or chronic disease. Blood samples were taken from patients and control groups after an overnight fasting period. Sera were isolated within 1 hour of blood sampling and stored at -20°C until further use.

Islet autoantibody determination:

ICA. Islet cell antibodies were detected by the indirect immunofluorescence technique. Frozen sections of monkey pancreas (EUROIMMUNE/ Germany) are incubated with diluted patients' samples(1:10). If the reaction is positive, specific antibodies of classes IgA, IgG and IgM attach to the tissue antigens. In a second step, the attached antibodies are stained with fluorescein- labeled anti- human IgG antibodies (EUROIMMUNE/ Germany) and made visible with fluorescence microscope (Figure a).

Figure (a): Primate pancreas: antibodies against islet . A fluorescent microscope image of positive result of Islet cell antibodies.(A= islet cells coated with fluorescein- labeled anti-human IgG antibodies).



GADA. Glutamic acid decarboxylase antibodies were detected by ELISA, human recombinant glutamic acid decarboxylase, isoform GAD65, was used for coating the microplate and preparation of the biotenylated GAD. If the sample is positive, specific antibodies bind to the GAD. Bound antibodies are able to act divalently and form a bridge GAD, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme – labeled avidin which is capable of promoting a color reaction. The upper limit of the normal range (cut-off value) recommended by (EUROIMMUNE/ Germany) is 10 international per milliliter (IU/ml), so results above 10 (IU/ml) consider positive.

IA-2A. Insulinoma-associated antigen 2 antibodies were detected by ELISA, with a test principle and procedure similar to that of GADAs. The upper limit of the normal range

(cut-off value) recommended by (EUROIMMUNE/ Germany) is 20 international per milliliter (IU/ml), so results above 20 (IU/ml) consider positive.

Cytokines Determination: Concentrations of Th1 cytokines [interleukin-2 (IL-2) , interferon gamma (IFN-γ) and tumor necrosis factor-β (TNF-β)], and concentrations of Th2 cytokines [Interlukin-4(IL-4), Interlukin-5(IL-5)and Interlukin-10(IL-10)] in sera of study groups were measured by enzyme linked immunosorbent assay (ELISA) with commercial kits of BioSource ,Belgium in accordance to manufacturer instructions. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of the interleukin being measured . According to the protocol developed by BioSource, the lower limits of detection for the individual assays are as follows: IL-2, 1.16 U/ml; IFN-γ, 5 U/ml ; TNF- β, 10 pg/ml ; IL-4, 0.2 pg/ml ; IL-5, 5 pg/ml; and IL-10, 1 pg/ml.

Statistical Analysis: All values were expressed as mean ± SD. Statistical analyses were done using the Student's t-test was used to assess differences between study groups. The level of significance was set at $P < 0.05$.

RESULTS:

Of 56 patients who received a diagnosis of type 1 diabetes, 15 patients (26.8%) had no detectable ICA, GADA, or IA-2. Thirteen (23.2%) patients were positive for one Islet autoantibody type (6 for ICA, 7 for GADA), and 28 (50%) patients had detectable serum levels of at least two autoantibodies [(GADA and ICA) or (GADA and IA-2A) or (ICA and IA-2A)] (Table 1). The three subgroups did not differ significantly for mean age or distribution of sex.

Of the three Th1 cytokines measured in this study (IL-2,INF-γ and TNF-β) , both INF-γ and TNF-β showed an increase when the patient had islet autoantibodies. The mean concentration of both INF-γ and TNF-β decrease in the absence of detectable islet autoantibodies (23.9 ± 3.2pg/ml and 2.3 ± 1.1U/ml) respectively versus a significant elevation in both cytokines concentrations in the presence of either one autoantibody (50.5 ± 5.9pg/ml and 13.8 ± 10.9 U/ml) respectively ($P < 0.05$) or two- three autoantibodies (63.9 ± 5.4 pg/ml and 13.7 ± 5.5 U/ml)) respectively ($P < 0.05$) as it shown both in table 2 and figures 2 and 3.While there was no significant correlation between IL-2 and autoantibodies status(table 2 and figure 1)

In the other hand neither of the three Th2 immunoregulatory cytokines concentrate significantly ,increased (54.4 ± 1.4), with the presence of Islet autoantibodies in IL-4 concentrat in positive autoantibodies patients than in negative autoantibodies which in turn showed higher concentration than control group (Table 2 and Fig.4,5,6).

DISCUSSION:

D.M Type 1 is a chronic inflammatory disease. There is, however, abundant evidence in animal models with spontaneous autoimmune diabetes "the non-obese diabetic (NOD) mouse and the BioBreeding (BB) rat" and to a lesser extent in humans with T1D that Islet β-cell destruction results from a disorder of immunoregulation [19]. By this it is meant that genetic factors (particular alleles of the human leukocyte antigen [HLA] [20] and other genes like insulin gene [21] andIL2RA (Allelic variation in the interleukin (IL)-2 receptor-_ gene) [22], together with environmental factors (possibly enteroviral agents) [23], predispose an individual to develop pathogenic Islet β-cell autoreactive T cells . These autoreactive T cells are believed to dominate over protective regulatory T cells when the latter fail to develop adequately, based on the individual's lack of possession of particular protective HLA and other genes, and possibly lack of exposure to protective environmental agents. The dominance of autoreactive T cells over regulatory T cells then would lead to Islet inflammation (termed insulitis), where Islets are infiltrated by macrophages, and CD4+ and CD8+ T cells that specifically destroy β-cells [24].

It was estimated that the presence of ICA and GADA at diagnosis of diabetes improves the classification of diabetes [25] and predicts the future need of insulin in young adults [26]. In humoral autoimmunity in T1D, the detection of islet autoantibodies and the examination of their associations with genetic factors and cellular autoimmunity constitute major areas in both basic research and clinical practice [27].

In current study, IL-2, (TNF)-β and INF-γ were studied as Th1 proinflammatory cytokines and IL-4, IL-5, IL-10 as Th2 immunoregulatory cytokines. There was an obvious domination of proinflammatory cytokines over immunoregulatory cytokines in diabetic patients. A positive association was found between the presence of Islet autoantibodies and two of the proinflammatory cytokines (TNF-β and INF-γ) while there was no correlation between Islet autoantibodies and immunoregulatory cytokines. This explains the concept that human

autoimmune diabetes is associated with an imbalance between the up-regulated Th1 and the down-regulated Th2 arms of the immune system. Evidence has been accumulating that the Islet autoantibody status at diagnosis of type1 diabetes reflects disease quality, in that patients with Islet autoantibodies exhibit faster loss of endogenous β-cell function during the next years [28,29]. In addition, multiple autoantibody positivity and titer seem relevant [30,31,32]. It therefore seems relevant that multiple Islet autoantibody-positive patients can be clearly distinguished from autoantibody-negative patients on the basis of systemic cytokine/chemokines concentrations [33].

Many studies support the result of the current study by measuring different kinds of cytokines of both Th1 and Th2 subsets of T-cells like TNF α. [34], IP-10 [35], INF γ [36]. But most of these studies did not compare between negative and positive Islet autoantibodies [37] making us unable to compare our complete results with other studies.

The communication between Th1 and Th2 cytokines such as IL-2 and IL-4 is complex; they may act either in synergism or opposition in promoting lymphocyte proliferation and differentiation according to the interaction between the timing of their secretion, their relative concentrations and the experimental system used [38]. This suggests that the pattern of secretion of these cytokines over time, as well as their total secretion, may be significant parameters in evaluating the relative function of the Th1/Th2 arms of the immune system rather than the limited determination of peak cytokine levels [39].

The association of Islet autoantibody status and cytokine/ chemokine levels in serum provides further evidence for the clinical relevance of systemic concentrations of immune mediators. Cytokines are monitoring disease activity as has been reported for diabetes development in autoimmune diabetic NOD mice [40]. Similarly, cytokine and chemokine levels may be useful for predicting the loss of residual C-peptide secretion [41].

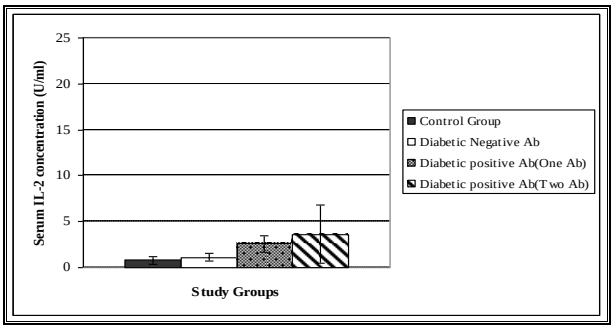


Fig.1: Serum Interleukin-2 concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

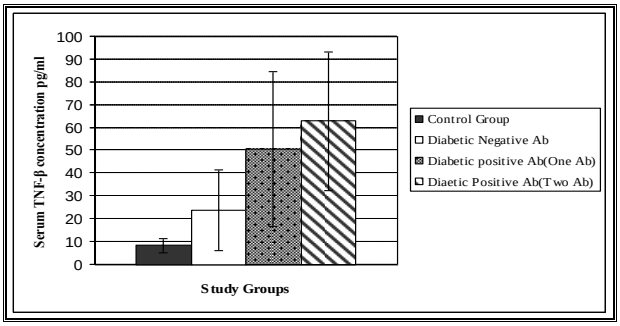


Fig.2: Serum TNF-β concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

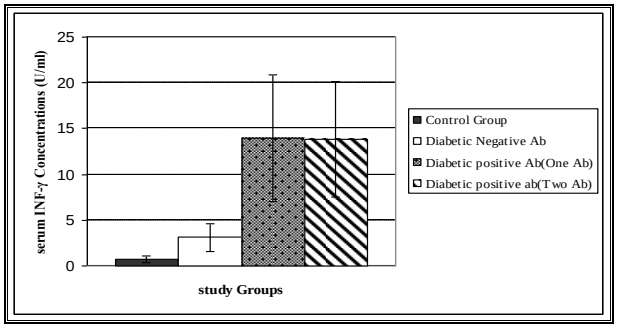


Fig.3: Serum INF-γ concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

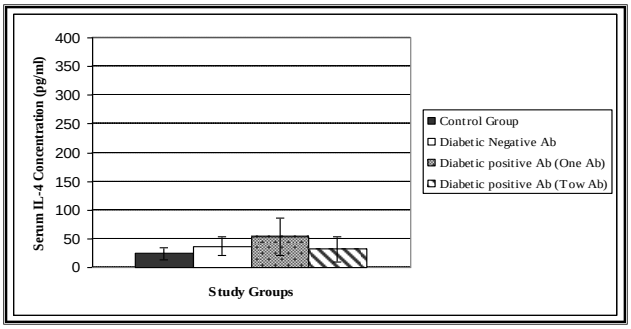


Fig.4: Serum Interleukin-4 concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

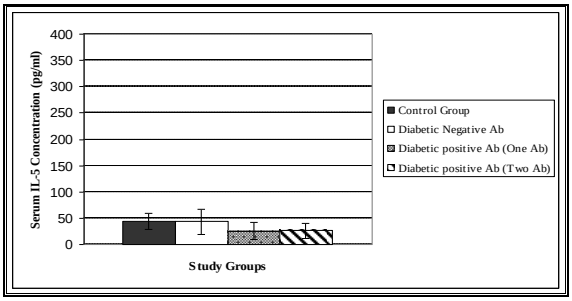


Fig.5: Serum Interleukin-5 concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

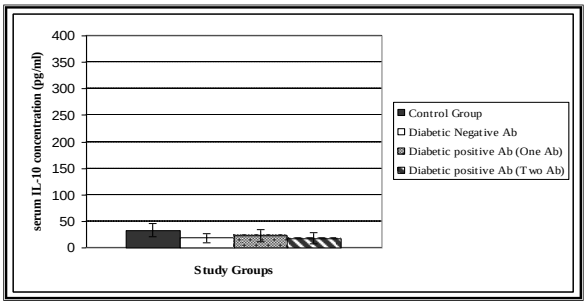


Fig.6: Serum Interleukin-10 concentrations in study groups. The bar represents the mean. Vertical line extends between ±2 SD.

Table 1: Islet Autoantibody status in patients

Positive Islet Autoantibodies				Negative Islet Autoantibodies	Total
Single autoantibody			Two or more autoantibodies		
ICA	GADA	IA-2			
6(10.7%)	7(12.5%)	-----	28 (50%)	15(26.8%)	100%

Table 2: Correlation between Cytokines concentrations and Islet Autoantibody status [Values are (Mean ±SD)].

	Control Group	Diabetic Negative Autoantibodies	Diabetic Positive Autoantibodies (one Ab)	Diabetic Positive Autoantibodies (Two Abs)
				Th1 Cytokines
IL- 2	1.2 ± 0.3	1.9 ± 0.4	3.0 ± 0.6	4.6± 2.3
TNF- β	8.9 ± 0.6	23.9 ± 3.2	50.5 ± 5.9*	63.9 ± 5.4*
INF-γ	6.7 ± 0.3	2.3 ± 1.1	13.8 ± 10.9*	13.7 ± 5.5*
				Th2 Cytokines
IL-4	36.9 ± 0.5	39.1 ± 0.7	54.4 ± 1.4	32.6 ± 0.4
IL-5	45.5 ± 0.7	45.5 ± 1.1	23.9 ± 0.8	26.2 ± 0.6
IL-10	32.6 ± 0.6	19.6 ± 0.4	23.9 ± 0.5	17.4 ± 0.5

* Significant correlation P <0.05.

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