

**ROLE OF CYTOKINES (INF- $\gamma$ , IL-6, IL-10) IN THE PATHOGENESIS OF  
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**ABSTRACT**

Type I diabetes mellitus is an autoimmune disease; it is one of most serious and common metabolic disorders in children and adolescents, in which the body immune system destroys the insulin releasing beta cells. Thus, the body cells cannot absorb glucose. Cytokines play a major role in the pathogenesis of autoimmune type I diabetes and in the initiation of  $\beta$ -cell damaging process. The objective of this study is to get more understanding about the role of cytokines in the development of beta cell destruction and immune state in T1DM patient, by measuring the IFN- $\gamma$ , IL-6 and IL-10. A total of sixty patients with long standing autoimmune T1DM (diagnosed more than one year) were included in the study. Twenty eight healthy control subjects had the measurement of serum IL-6, IL-10 and IFN- $\gamma$  by enzyme linked immunosorbent assay (ELISA) technique. Higher serum levels of IL-6 and IFN- $\gamma$  were observed in the patients (224.02, 394.34 pg/ml respectively) compared to controls (164.65, 264.07 pg/ml respectively). The statistical analysis showed a significant difference between patients and controls ( $P= 0.0001$ ,  $0.017$  respectively). A difference of the mean IL-6 concentration appears between patients in two age groups in T1DM age (1 -10 y) ( $P= 0.085$ ). No significant differences appear in the mean between patients and controls for BMI and age ( $p= 0.865$ ,  $0.149$  respectively). No significant difference appear in the serum level IL-6, IL.10 and IFN- $\gamma$  between patients divided into two group according HbA1c (T1DM controlled) and (T1DM uncontrolled) ( $p=0.604$ ,  $0.423$ ,  $0.590$ ). In long standing patients with autoimmune type I diabetes mellitus, clinical significant elevation in serum levels of IFN- $\gamma$  and IL-6 while IL-10 serum level begin decline. A difference of mean IL-6 concentration appears between patients in both age groups T1DM age (1 -10 y). No significant differences appear in the mean serum concentrations of IFN- $\gamma$ , IL.10 and IL-6 between patient and control for BMI and age. No significant difference appear in the serum level IFN- $\gamma$ , IL-10 and IL-6 between patients divided into two groups according HbA1c (T1DM controlled) and (T1DM uncontrolled).

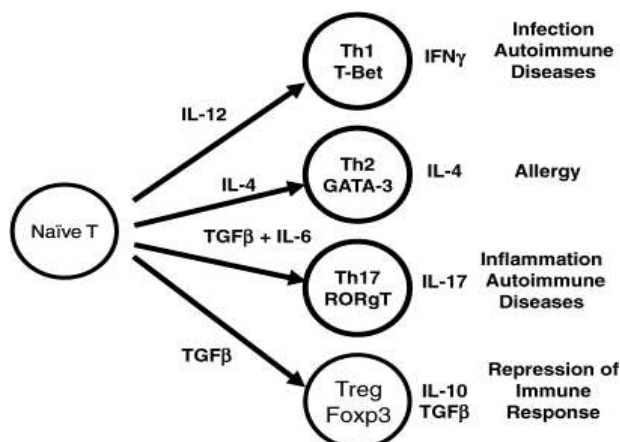
**KEYWORDS:****INTRODUCTION**

Diabetes mellitus is a chronic metabolic disease described by elevation in levels of blood sugar over time leads to severe damage to the heart, blood vessels, kidneys, eyes, and nerves.<sup>[1]</sup> Type I diabetes mellitus (T1DM) is an autoimmune disease characterized by beta cell destruction, associated with cellular infiltration and inflammatory responses in the islets of Langerhans. The cellular components of this infiltrate include monocytes, macrophages, CD4+ and CD8+ T cells and the balance between Th1 and Th2 cells is crucial in the pathogenesis of this disease.<sup>[2,3,4]</sup> T cells can directly kill beta cells via cell-to-cell contact, through a cytotoxic process, but they can also influence their destruction through other factors, including the release of pro-inflammatory cytokines, granzyme B, or perforin, and possibly signaling through pathways of programmed cell death.<sup>[5]</sup>  $\beta$ -Cell death in the course of insulinitis is probably caused by direct contact

with activated macrophages and T-cells, and/or exposure to soluble mediators secreted by these cells, including cytokines, nitric oxide (NO), and oxygen free radicals.<sup>[6]</sup>

Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of immune system.<sup>[7]</sup> Cytokines are a category of signaling molecules that mediate and regulate immunity, inflammation and hematopoiesis.<sup>[8]</sup> Cytokines have been reported to be involved in the immunopathology of several autoimmune diseases including Type I diabetes.<sup>[9]</sup> There is evidence that cytokines could have a direct role in beta-cell death.<sup>[10]</sup> Interleukin-6, also produced by macrophages, is a key mediator of multiple inflammatory and immune responses.<sup>[11]</sup> IFN- $\gamma$ , produced by activated T lymphocytes, activates macrophages enhances class I Major Histocompatibility Complex (MHC) antigen expression and induces class II

expression in combination with TNF on normal cultured human islet cells IFN- $\gamma$  also enhances TNF induced human islet cells cytotoxicity.<sup>[12]</sup> IL-10 suppresses both cytokine production and antigen-specific proliferation of cultured clones of TH1 cells when they are activated in an accessory cell-dependent manner.<sup>[13]</sup> These studies indicate that cytokines may have role in the pathogenesis of Type I diabetes.



**Figure 1.1:** Cytokine and role in autoimmune disease.<sup>[14]</sup>

## METHODOLOGY

### I. Design of study

#### 1. Patients Group

Sixty patients diagnosed with autoimmune type I diabetes mellitus (33 males and 27 females).

#### 2. Control Group

Twenty eight healthy subjects as control (13 males and 15 females).

### II. Data Collection

#### 1. Inclusion criteria

1. Diagnosis autoimmune type I diabetes mellitus (GAD +ve)
2. Age (1 to 16 years)
3. Long standing DM since more than 1 year

#### 2. Exclusion criteria

Excluded any other autoimmune disease and inflammation.

### III. Sample collection

This study was achieved by collecting 88 blood samples from 60 patients (33 males and 27 females) with autoimmune type I Diabetes Mellitus and 28 subjects as control (13 males and 15 females).

Patients were admitted to Al-Faiha Specialized Diabetes, Endocrine and Metabolic center (FDEMC) in Basrah City/ Iraq from 21/11/ 2017 to 1/5/2018. The study sample ages ranged from (1 – 16) years old.

### 1. For cytokines profile

A sample of 3 ml of blood was collected in plane tube then let stand for about (20 – 30) minutes to clot formation and centrifuged by using centrifuge for about (5- 10) minutes on speed of 2500 rpm. Then fresh non hemolysis serum was collected and kept in deep freeze (-20 0C). Serum was collected in two eppendorf tubes.

### 2. For HbA1c

A sample of 3 ml of whole blood was collected in a vacuum collection tube containing EDTA make directly or storage up to 4 days at (2-8 c) or 1 day at room temperature (15-30 c).

### IV. Statistical analysis

Statistical analysis was performed by using (SPSS) version (20) and all data presented using chi-square and t tests.  $p > 0.05$  was considered to be significant statistically.

### V. Subject

Sixty patients diagnosed with autoimmune type I diabetes mellitus (33 males and 27 females) and twenty eight healthy subjects as control (13 males and 15 females). The patients were divided into two groups depending on glycated hemoglobin (HbA1c) as six controlled DM patients (2 males and 4 females) and uncontrolled DM (25 males and 29 females). Moreover, they were divided into two groups depending on the duration of disease as 47 patients (1 to 5 years) and 13 patients (6 to 11 years).

## VI. RESULTS AND DISCUSSION

### 1. Cytokines profile(IL-6, IL-10, IFN- $\gamma$ ) in autoimmune T1DM patient and control

In table (3.1), higher serum levels of IFN- $\gamma$  and IL-6 were observed in the patients (224.02, 394.34 pg/ml respectively) compared to controls (164.65, 264.07 pg/ml respectively).

The statistical analysis revealed a significant difference between patients (T1DM) and controls ( $P= 0.0001, 0.017$  respectively); whereas for serum levels of IL-10 in patients (156.57 pg/ml) compared to controls (142.08 pg/ml), the statistical analysis revealed no statistically significant difference between patients (T1DM) and controls ( $P= 0.141$ ).

**Table 3.1:** Comparison of the cytokine profile between autoimmune T1DM and control healthy subjects.

Cytokine	Autoimmune T1DM N=60	Control N=28	P value
IL-6	394.34 $\pm$ 381.59	264.07 $\pm$ 108.54	0.017
IL-10	156.57 $\pm$ 38.72	142.08 $\pm$ 49.98	0.141
IFN- $\gamma$	224.02 $\pm$ 68.87	164.65 $\pm$ 44.71	0.0001

Cytokines have the main role in the immuno pathology of several autoimmune diseases including Type I diabetes mellitus.<sup>[15]</sup> There is support from in vitro

studies that cytokines have a direct role in promoting pancreatic Beta cell death.<sup>[16]</sup>

### I. Interleukin 6

IL-6 is an inflammatory cytokine secreted from T-cells, B-cells and several non-lymphoid cells including macrophages, endothelial cells, fibroblast and bone-marrow stromal cells.<sup>[17]</sup> The present results indicate a high level of serum IL-6 in autoimmune T1DM patients as compared to controls; this added a support that the disease is an immune inflammatory disorder. This result is in common with other reports.<sup>[18]</sup> IL-6 is a potent inducer of hepatic acute phase protein (C-reactive protein). Elevated C-reactive protein level, detected in infants and young children before the onset of autoimmune T1DM,<sup>[19]</sup> may provide an additional marker for risk of progression to T1DM. IL-6 is a "co-stimulatory signal" for T-cell activation made by certain antigen presenting cells (APCs), and previously known as B-cell differentiation factor. It acts on most cells, but is particularly important in inducing B-cells to differentiate to antibody-forming cells.<sup>[17]</sup> Mechanically, IL-6 may exert its effect by inducing a condition of increased energy spending in the islet, through elevated glucose oxidation and oxygen uptake accompanied by a partial inhibition of the glucose stimulated insulin release and lowering the islet cellular ATP contents.<sup>[20]</sup>

### II. IFN- $\gamma$

IFN- $\gamma$  was originally identified by its antiviral activity and was discriminated from IFN- $\alpha$  and IFN- $\beta$  by its liability at pH 2.0; a property often used as a simple method of identification.<sup>[21]</sup> The current results confirmed that serum IFN- $\gamma$  concentrations were elevated in patients with autoimmune T1DM compared to its concentration in the healthy controls. These data were in common with other studies which stated that inflammatory cytokine IFN- $\gamma$  may play a significant role in the pathogenesis of T1DM, and its concentration was higher in T1DM patients.<sup>[22]</sup> Many studies largely support the concept that  $\beta$ -cell destruction is associated with increased expression of inflammatory cytokines (IL-1, TNF- $\alpha$  and IFN- $\alpha$ ) and Th1 cytokines (IFN- $\gamma$ , TNF- $\beta$ , IL-2) and IL-12.<sup>[23]</sup> Mechanically, pro-inflammatory and Th1 cytokines including IFN- $\gamma$  induce and accelerate  $\beta$ -cell destruction through direct and indirect mechanisms. Directly, Th1 cytokines including IFN- $\gamma$  exerted their property primarily at the level of macrophages, enhancing infiltration of these cells in the islet, thus accelerating  $\beta$ -cells destruction throughout the release of performed de novo synthesized cytotoxic mediators (nitric oxide, oxygen radicals... etc.).<sup>[24]</sup> or induced T-cells infiltrate the islets (MHC class I restricted CD8 + T-cells) because IFN- $\gamma$  and TNF up regulate expression of MHC class I, which in conjugation with autoreactive T-cells could bring about extensive tissue damage on rodents and human  $\beta$ -cells.<sup>[23,25]</sup>

### III. IL 10

In this study, it can be noted that IL-10 has no clinical significance when comparing long standing autoimmune T1DM patients with healthy controls, where as other studies documented elevated IL-10 in newly diagnosed T1DM patients.<sup>[26]</sup>

Early peak of serum IL-10 level was observed initially, but the continued loss of IL-10 until progression toward diabetes was observed, confirming the fact that IL-10 was essential for an early phase of diabetes.<sup>[27]</sup> In other studies, it was noted that the level of IL-10 in long standing diabetes was lower than healthy control groups.<sup>[28,29]</sup> This confirms the imbalance in immune system of diabetic patients.

### 2. Cytokines profile (IL-6, IL-10, IFN- $\gamma$ ) in autoimmune T1DM patients depending on glycated hemoglobin (HbA1c)

In table (3.2) the level of cytokines (IL-6, IL-10 and INF-G) profile is compared between the two groups depending on HbA1c as controlled T1DM. The level of HbA1c less than 7.5% observed the mean level of IL-6, IL-10 and IFN- $\gamma$  (313.76, 144.43, 210.03 pg/ml respectively) and uncontrolled T1DM HbA1c more than 7.5% (403.29, 157.92, 225.57 pg/ml respectively) noted that the higher level of IL-6 in uncontrolled T1DM.

The statistical analysis revealed no statistically significant difference between the two groups (P= 0.590, 0.423, 0.604 respectively).

**Table 3.2: Comparison of cytokine profile between controlled autoimmune T1DM and uncontrolled autoimmune T1DM.**

Cytokine	T1DM controlled N=6	T1DM uncontrolled N=54	P value
IL-6	313.76±147	403.29±339.05	0.590
IL-10	144.43±15	157.92±40.35	0.423
IFN- $\gamma$	210.03±76	225.57±68.58	0.604

The lack of a significant relationship between cytokines and glycated hemoglobin is in agreement with study.<sup>[30]</sup> In the current study, the mean serum level of IL-6 in uncontrolled T1DM is higher than controlled T1DM but this was not significant because the number of uncontrolled patients was very small.

Other studies showed that serum IL-6 concentrations were significantly higher in diabetic patients with a strong positive correlation with both the FBG and HbA1c. Hyperglycemia stimulates monocytes to secrete increased amounts of IL-6 via up regulation of protein kinase C (PKC), mitogen-activated protein kinase (p38 MAPK) and Nuclear factor (NF- $\kappa$ B) activity, leading to increased IL-6 transcription and release.<sup>[31,33]</sup> Other studies also showed higher IL-6 expression in T1DM patients with poor glycemic control (according to the

values of glycated hemoglobin HbA1c) when compared to control groups.<sup>[32]</sup>

### 3. Age, BMI and HbA1c in autoimmune T1DM patients and controls

Table (3.3) compares between healthy subjects and T1DM patients according the age, gender, BMI and HbA1c.

The mean of age in patients was (13.1 years) compared to controls (11.75 years). The statistical analysis revealed no statistical significant difference (0.149).

The mean of body mass index (BMI) in the patients was (19.23) compared to controls (18.82). The statistical analysis revealed no statistical significant difference (0.865).

The mean of blood level of HbA1c in the patients was (10.94%) compared to controls (4.88%). The statistical analysis revealed a high significant difference (T1DM) and controls (P= 0.0001).

**Table 3.3: Comparison of age, gender, BMI and HbA1c between healthy subjects and T1DM patients.**

	Control (healthy) N=28	T1DM patient N=60	P value
Age	11.75±3.5	13.1±3.4	0.149
BMI	18.82±3	19.23±3.4	0.865
HbA1c	4.88±0.66	10.94±2.5	0.0001

No significant difference in age, gender and body mass index (BMI) between healthy subjects and T1DM patients was noted. These results are agreement with other studies.<sup>[30]</sup>

And there is highly significant level of HbA1c in patients; this is because the patients have glycated haemoglobin more than the normal range.

### 4. Cytokines profile (IL-6, IL-10, IFN- $\gamma$ ) in autoimmune T1DM patients depending on duration of diabetes

In table (3.4), the levels of cytokines (IL-6, IL-10 and INF-G) profile were compared between two groups depending on duration of disease. With the duration of diabetes 1-5 years serum levels of IL-6, IL-10 and INF-G were 435.0, 158.9, 219.3 pg/ml respectively and with duration of diabetes 6-11 years the levels of IL-6, IL-10 and INF-G were 246.3, 148.1, 240.8 pg/ml respectively. The statistical analysis revealed no clinical significant difference between the two groups (P= 0.115, 0.380, 0.325 respectively).

**Table 3.4: Comparison between patients with T1DM depending on the duration of disease.**

Cytokine	Duration of diabetes (1-5 y)	Duration of diabetes (6-11 y)	P value
IL-6	435.0±418	246.3±116	0.115
IL-10	158.9±42	148.1±17	0.380
INF-G	219.3±67	240.8±73	0.325

According to duration of diabetes, the levels of these cytokines remain elevated, but the mean serum level of IL-6 in patients with diabetes duration from 6 to 11 years is less than that of the duration 1 to 5 years. This agrees with other studies.<sup>[26]</sup> Also this study confirms that the level of IL-6 begins to decline after 6 to 11 years. This means that pancreatic beta cell completely destruction. Other studies show there was a significant difference in the expression of IFN- $\gamma$  only between patients with more than 10 years of disease duration (P = 0.02).<sup>[34]</sup>

### CONCLUSIONS

In long standing patients with autoimmune type I diabetes mellitus, there is statistically significant elevation in serum levels of IFN- $\gamma$  and IL-6 while IL-10 serum level begins to decline.

A difference in the mean IL-6 concentration level appears between patients in both age groups T1DM age. No significant differences appear in the mean serum concentrations level of IFN- $\gamma$ , IL-10 and IL-6 between patients and controls for BMI and age.

No significant difference appear in the serum levels IFN- $\gamma$ , IL-10 and IL-6 between patients divided into two groups according HbA1c (T1DM controlled and T1DM uncontrolled).

### RECOMMENDATIONS

1. Study the possible exposure to one or more environmental risk factors that can alter the immune system and cause demotion of beta cells like cow milk protein.
2. Considering the unknown long term safety profile of immunomodulatory agents such as anti-CD3 (T cell) or anti-CD 20 (B cell) antibodies and IL-21 neutralization as well as the transience of remission induced by some of these drugs, there is an urgent need for studies to define the clinical benefit of combining therapies targeting key pathways in the inflammatory pathogenesis of type I diabetes mellitus to increase safety and efficacy.
3. Study the association of type I diabetes with other autoimmune diseases like (thyroid disease, celiac disease, gastritis, Addison's disease).
4. Study the role of immune drugs that suppress immune response and can modulate T1DM.
5. Comparison study between autoimmune T1DM as duration of disease group more than 11 years compared with group less than 5 years by determining the level of cytokines.

6. Need study compares between autoimmune T1DM according to HbA1c as controlled and uncontrolled but more number of patients by determine cytokines profile.
7. Study comparing T1DM patients with complications to T1DM patients without by determining cytokines profile.

## REFERENCES

1. "About diabetes". World Health Organization. Archived from the original on 31 March 2014. Retrieved 4 April 2014. Parker, R. M., Ratzan, S. C., & Lurie, N. (2003). Health literacy: a policy challenge for advancing high-quality health care. *Health affairs*, 22(4), 147-153.
2. Gomes, K. B. (2017). IL-6 and type I diabetes mellitus: T cell responses and increase in IL-6 receptor surface expression. *Annals of translational medicine*, 5(1).
3. Badami, E., Sorini, C., Coccia, M., Usuelli, V., Molteni, L., Bolla, A. M.,... & Falcone, M. (2011). Defective differentiation of regulatory FoxP3+ T cells by small-intestinal dendritic cells in patients with type I diabetes. *Diabetes*, 60(8), 2120-2124.
4. Vendrame, F., Cataldo, D., Ciarlo, L., Umland, O., Misasi, R., & Dotta, F. (2012). In Type I Diabetes Immunocompetent Cells are Defective in IL-16 Secretion. *Scandinavian journal of immunology*, 75(1), 127-128.
5. Khadra, A., Pietropaolo, M., Nepom, G. T., & Sherman, A. (2011). Investigating the role of T-cell avidity and killing efficacy in relation to type I diabetes prediction. *PloS one*, 6(5), e14796.
6. Cnop, M., Welsh, N., Jonas, J. C., Jörns, A., Lenzen, S., & Eizirik, D. L. (2005). Mechanisms of pancreatic  $\beta$ -cell death in type I and type 2 diabetes: many differences, few similarities. *Diabetes*, 54(suppl 2), S97-S107.
7. Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews Immunology*, 1(2), 135.
8. Fantuzzi, G., & Faggioni, R. (2000). Leptin in the regulation of immunity, inflammation, and hematopoiesis. *Journal of leukocyte biology*, 68(4), 437-446.
9. Dinarello, C. A., & Mier, J. W. (1987). Lymphokines. *New England Journal of Medicine*, 317(15), 940-945.
10. Palmer, J. P., Helqvist, S., Spinas, G. A., Mølviq, J., Mandrup-Poulsen, T., Andersen, H. U., & Nerup, J. (1989). Interaction of  $\beta$ -cell activity and IL-1 concentration and exposure time in isolated rat islets of Langerhans. *Diabetes*, 38(10), 1211-1216.
11. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y.,... & Kishimoto, T. (1990). A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *The EMBO journal*, 9(6), 1897-1906.
12. Hussain, M. J. (1996). Role of cytokines in the pathogenesis of type I diabetes (Doctoral dissertation, Brunel University School of Health Sciences and Social Care PhD Theses).
13. Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W., & O'Garra, A. (1991). IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *The Journal of Immunology*, 146(10), 3444-3451.
14. (Hirano, T. (2010). Interleukin 6 in autoimmune and inflammatory diseases: a personal memoir. *Proceedings of the Japan Academy, Series B*, 86(7), 717-730.)
15. Mier, J. W., Dinarello, C. A., Atkins, M. B., Punsal, P. I., & Perlmutter, D. H. (1987). Regulation of hepatic acute phase protein synthesis by products of interleukin 2 (IL 2)-stimulated human peripheral blood mononuclear cells. *The Journal of Immunology*, 139(4), 1268-1272.
16. Palmer, J. P., Helqvist, S., Spinas, G. A., Mølviq, J., Mandrup-Poulsen, T., Andersen, H. U., & Nerup, J. (1989). Interaction of  $\beta$ -cell activity and IL-1 concentration and exposure time in isolated rat islets of Langerhans. *Diabetes*, 38(10), 1211-1216.
17. Owen, J. A., Punt, J., & Stranford, S. A. (2013). *Kuby immunology* (p. 692). New York: WH Freeman.
18. Hammed, I. K. (2012). Serum Interleukin-6 level in children with type I diabetes mellitus. *Journal of the Faculty of Medicine*, 54(3), 228-229.
19. Chase, H. P., Cooper, S., Osberg, I., Stene, L. C., Barriga, K., Norris, J.,... & Rewers, M. (2004). Elevated C-reactive protein levels in the development of type I diabetes. *Diabetes*, 53(10), 2569-2573.
20. SANDLER, S., BENDTZEN, K., Eizirik, D. L., & WELSH, M. (1990). Interleukin-6 affects insulin secretion and glucose metabolism of rat pancreatic islets in vitro. *Endocrinology*, 126(2), 1288-1294..
21. De Weerd, N. A., & Nguyen, T. (2012). The interferons and their receptors—distribution and regulation. *Immunology and cell biology*, 90(5), 483-491.
22. Özer, G., Teker, Z., Çetiner, S., Yılmaz, M., Topaloglu, A. K., Önenli-Mungan, N., & YüKSEL, B. (2003). Serum IL-1, IL-2, TNF $\alpha$  and INF $\gamma$  levels of patients with type I diabetes mellitus and their siblings. *Journal of Pediatric Endocrinology and Metabolism*, 16(2), 203-210.
23. Kukreja, A. and Maclaren, N. (1999) Autoimmunity and diabetes. *J. Clin. Endocrinol. Metab.*; 84: 4371-4378.
24. Karlsen, A. E., Pavlovic, D., Nielsen, K., Jensen, J., Andersen, H. U., Pociot, F.,... & Nerup, J. (2000). Interferon- $\gamma$  induces interleukin-1 converting enzyme expression in pancreatic islets by an interferon regulatory factor-1-dependent mechanism. *The Journal of Clinical Endocrinology & Metabolism*, 85(2), 830-836.
25. Seewaldt, S., Thomas, H. E., Ejrnaes, M., Christen, U., Wolfe, T., Rodrigo, E.,... & von Herrath, M. G. (2000). Virus-induced autoimmune diabetes: most

- beta-cells die through inflammatory cytokines and not perforin from autoreactive (anti-viral) cytotoxic T-lymphocytes. *Diabetes*, 49(11), 1801-1809.
26. Saleh, E. M. (2009). Cytokines Profile in Newly Diagnosed Children with Type I Diabetes Mellitus. *Journal of the Faculty of Medicine*, 51(3), 295-299.
  27. Roep, B. O., Hiemstra, H. S., Schloot, N. C., De Vries, R. R. P., Chaudhuri, A., Behan, P. O., & Drijfhout, J. W. (2002). Molecular mimicry in type I diabetes: immune cross-reactivity between islet autoantigen and human cytomegalovirus but not Cocksackie virus. *Annals of the New York Academy of Sciences*, 958(1), 163-165.
  28. Sia, C. (2005). Imbalance in Th cell polarization and its relevance in type I diabetes mellitus. *The Review of Diabetic Studies*, 2(4), 182.
  29. Jin shui he and pu-song xia (2014)role of immune dysfunction in pathogenesis of T1DM in children
  30. Munther J Hussain,(1996) Role of cytokines in the pathogenesis of type I diabetes
  31. G. Kavitha, G.Ramani, Priya K Dhass, Rita Mary Aruna. (2011)Oxidative stress,interleukin -6 and atherogenic index of plasma in diabetic nephropathy.*International journal of applied biology and Pharmecutical technology*; Volume: 2: Issue-2:211-217
  32. Hundhausen C, Roth A, Whalen E, et al. (2016)Enhanced T cell responses to IL-6 in type I diabetes are associated with early clinical disease and increased IL-6 receptor expression. *Sci Transl Med*;8:356ra119. 10.1126/scitranslmed.aad9943
  33. Hammed, I. K. (2012). Serum Interleukin-6 level in children with type I diabetes mellitus. *Journal of the Faculty of Medicine*, 54(3), 228-229.
  34. Vaseghi, H., & Jadali, Z. (2016). Th1/Th2 cytokines in Type I diabetes: Relation to duration of disease and gender. *Indian journal of endocrinology and metabolism*, 20(3), 312.