

**COMPARATIVE STUDY OF SOME IMMUNE INDICATORS IN THE SERUM OF PATIENTS WITH TYPE 1 DIABETES**Dhuha Adnan Neamah<sup>\*1</sup>, Zainab A. Fadhil<sup>2</sup>, Fyaa Raad Yaha<sup>3</sup> and Adnan Neamah Abdulridha<sup>4</sup><sup>1,2</sup>Department of Biology, College of Education for Pure Science - University Diyala-Iraq.<sup>3</sup>Al- Ahwaz intermediate School for boys /Diyala General Directorate of Education-Iraq.<sup>4</sup>Bilad Alrafidain University College/Diyala – Iraq.

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**ABSTRACT**

Type 1 diabetes (T1DM) is a chronic autoimmune disease characterized by the destruction of pancreatic  $\beta$ -cells, that produce insulin in specific areas of the pancreas, known as islets of Langerhans, leading to insulin deficiency. The study aimed to compare levels of IL-6, CRP and Immunoglobulin (IgM, IgG, IgA) in the serum of patients and healthy controls, and study the influence of age and gender on the prevalence of T1DM. The present study was conducted in Baquba Teaching Hospital / Diyala province for the time period from January and the last day of February 2023. A blood sample was taken from 50 volunteers (30 T1DM patients and 20 healthy people) aged between (1-75) years. Serum concentrations of IL-6 indicators within samples were investigated by Sandwich ELISA, the latex agglutination test was used to examine CRP, and measuring the concentration of immunoglobulin (IgA, IgM, IgG) in the serum of patients and healthy people. Results of the present study showed that the (1-20) years age group scored the highest percentage (43.3%) of patients ( $p < 0.05$ ). The difference between males and females was not significant ( $p > 0.05$ ). The results also showed an increase in IL-6 levels in T1DM patients ( $71.36 \pm 25.09$ ) than healthy ( $17.11 \pm 8.84$ ) with a significant difference ( $p < 0.05$ ). There was also a significant difference ( $p < 0.05$ ) in CRP positivity and negativity, between T1DM patients compared to healthy people. The results also showed raised levels of IgG, IgM, IgA in T1DM patients ( $2116.22 \pm 871.70$ ,  $2035.27 \pm 901.18$ ,  $1894.13 \pm 622.33$ ) than healthy ( $1916.35 \pm 791.96$ ,  $242.50 \pm 103.01$ ,  $432.14 \pm 189.82$ ) with a significant difference ( $p < 0.05$ ). The study found that there are significant differences in the age factor, while there are no clear differences in the gender factor, and that sick samples had higher amounts of IL-6, CRP and immunoglobulin (IgM, IgG, IgA) than healthy control samples.

**KEYWORDS:** Type 1 diabetes, interleukin-6, C-reactive protein, Immunoglobulin.**INTRODUCTION**

T1DM is a chronic autoimmune illness defined by the death of pancreatic  $\beta$ -cells, which generate insulin in particular parts of the pancreas known as islets of Langerhans, leading to insulin deficiency (Buchmann, et al., 2023). Worldwide, the incidence of T1DM is rising at a rate of 3–5% per year, and known genetic causes cannot explain this trend. (Ziegler, 2023). Activated T-cells and proinflammatory cytokines in the immune infiltration combine to cause programmed cell death (apoptosis) in the  $\beta$ -cells. (Hulme, et al., 2012). It is believed that the combination between environmental variables and T1DM susceptibility gene variations initiates the immune-mediated death of  $\beta$ -cells. (Hammed et al., 2012). As a result, the body suffers from a lack of insulin, which leads to high blood sugar, requiring lifelong external insulin therapy (Rodrigues Oliveira et al., 2023). There is an alternative treatment for insulin,

which is based on stem cells and has the ability to restore beta cell function and thus control blood sugar levels (Reynolds and Singh, 2023). Although this treatment has made great progress in pre-clinical studies, it has major limitations and is still in its early stages, in addition to As mentioned, there are other types of treatments such as gene therapy, immunotherapy and artificial pancreas (Singh et al., 2023).

One of the serious health consequences of T1DM is damage to small and large blood vessels and other vital organs, resulting in serious complications such as cardiovascular disease, eye and kidney disease, and limb amputation (Hallström et al., 2021). T1DM can lower both quality of life and major health implications. (Felton et al., 2023). T1DM is divided into two types: T1DM type A, which constitutes about 90% of the percentage of patients with T1DM, and the other type,

T1DM type B, which constitutes about 9% of affected patients (Cano-Cano *et al.*, 2023). There is another type of diabetes that affects children and is called Maturity onset diabetes of the young (MODY) occurs due to the presence of Islands of Langerhans in the pancreas. This type is associated with six different mutations and is not associated with autoimmunity, It constitutes a percentage 1-2% of infected children (Oliveira *et al.*, 2020). The study conducted by Kristiansen and Mandrup-Poulsen (2005), showed that there is a relationship between inflammatory mediators such as tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , and the family of cytokines IL-6 and IL-18, in causing both types of diabetes.

Interleukin-6 (IL-6) is a proinflammatory and pleiotropic cytokine expressed in numerous chronic inflammatory diseases. It has an influential role in autoimmune

diseases (Gotzmann, *et al.*, 2023). It plays an important role in metabolic control, notably homeostasis of glucose (Al-Hasso., 2023). As well as the role it plays in diabetes, by creating insulin resistance responsible for poor blood sugar control (Kalaivani, *et al.*, 2023). An increased risk of cardiovascular events and death has been linked to persistently elevated systemic IL-6; this correlation may be brought about by the endothelium's deposition of excess glucose, oxidized low-density lipoprotein (LDL-C), and cholesterol. (Holt, *et al.*, 2023). After tissue injury, T cells and macrophages release IL-6. (Kreiner *et al.*, 2022). Because of this, its physiological acute release in reaction to adverse stimuli triggers fever onset, the mobilization of acute-phase reactants (such C-reactive protein), the enhancement of leukocyte activity against pathogens, and the repair of physical injuries. (Myśliwska *et al.*, 2009).

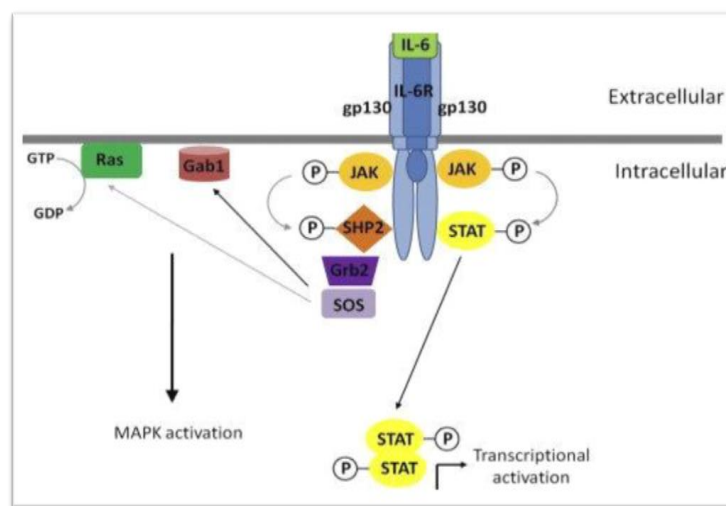


Figure 1: Diagram showing IL-6 signaling events (Al-Hasso., 2023).

An acute phase reactant plasma protein called a high C-reactive protein (CRP) is created in response to certain inflammatory stimuli. (Mouliou, 2023); (Bouayed *et al.*, 2022). It is considered an essential sign for diagnosing inflammation and predicting the development of autoimmune diseases, such as T1DM (Stanimirovic *et al.*, 2022). CRP is a risk factor for diabetes, and those who have the condition have greater CRP levels than those who do not. Moreover, systemic inflammation may contribute to the early stages of glucose metabolism degradation. (Kato *et al.*, 2019).

Immunoglobulin M (IgM) is the first antibody produced in the body to fight a new infection, as it represents the basis of the primary immune response and is characterized by its large size compared to other types (Platt and Cascalho, 2015). It is found mainly in the blood and lymphatic fluid, as its percentage reaches about 10% of the total immune globulins in normal human serum (Pomorska-Mól *et al.*, 2020). Immunoglobulin G (IgG) is a glycoprotein, characterized by its superior ability to spread between tissues (Xu *et al.*, 2020). It can spread inflammation, through its

binding to receptors that activate innate immune cells, and the opposite happens when it is given in high doses intravenously. (Yang *et al.*, 2023). IgG can also exert similar paradoxical properties on glucose metabolism, elicit insulin resistance, and conversely, when administered in high doses, enhance insulin sensitivity in a diabetic mouse model (Prasad *et al.*, 2013). An increased concentration of IgG has been found in the serum of diabetic patients, which strongly suggests the presence of advanced end products associated with it (Lipchik *et al.*, 2021). When the mechanisms fail to prevent the activation and differentiation of B cells, harmful antibodies (self-IgG and IgM) are secreted to form autoimmune diseases, such as T1DM (Amendt and Yu, 2023). Immunoglobulin A (IgA) is a glycoprotein, which is the most produced in the body. The binding of IgA during infection or inflammation causes immune cells to become activated, which in turn can trigger pro-inflammatory responses. These reactions are thought to happen when the proportion of IgA is high. (Thakkar *et al.*, 2022). The study conducted by Abouali *et al.* (2020), for patients with T1DM showed that there is a decrease in the level of IgA [Selective IgA deficiency (SIgAD)],

Uncertainty surrounds the significance of increased serum IgA in the etiology of T1DM and other autoimmune illnesses for which it has been linked.

Objective of this review is to demonstrate the contribution of IL-6 and CRP in T1DM etiology.

**MATERIAL AND METHODS**

**Samples collection**

The present study was conducted in Baquba Teaching Hospital / Diyala province for the time period from January and the last day of February 2023. Participants who visited the Baquba Educational Hospital along with those who had symptoms of T1DM had thirty. Specimens of blood taken, and a consulting physician at the consulting units/Baquba Training Hospital examined and made an assessment. Twenty specimens of blood were taken from healthy individuals and used as the control group. The healthy volunteers and patients varied in age between 1 to 75.

**Methods**

To separate the serum, five ml of human blood with gel tube were spun at 4000 rpm for 5 minutes. To conduct a group of immunological tests, serum concentrates of IL-6 indicators within samples were investigated by Sandwich Enzyme-linked immune-sorbent assay (ELISA) (CUSABIO-company) (Baselli et al., 2023). Latex agglutination test was used to examine CRP (Komoriya et al., 2010). The concentration of immunoglobulin (IgA, IgM, IgG) was also measured by single radial immunodiffusion test.(Mancini et al., 1965).

**Statistical analysis**

The immunoglobulin IgG, IgM, IgA and IL-6 were appeared as Mean±SD, with *student t test* used to determine the differences significance (patients versus healthy). Demographic features (age and gender) were appeared as frequencies and percentages, and differences among percentages were detected by *Pearson-Chi-square* test.  $P \leq 0.05$  was applied to detect significant differences. Data of our research were systemic by SPSS version 25.0 and Graph pad prism version 6 statistical software programs.

**RESULTS**

**1. Demographic features of participants**

Data of present study showed the 1 to 20 years age group scored highest percentage (43.3%) of patients 0( $p < 0.05$ ). the difference between males than females were no significant ( $p > 0.05$ ) (table 1).

**Table 1: Demographic Features of T1DM patients.**

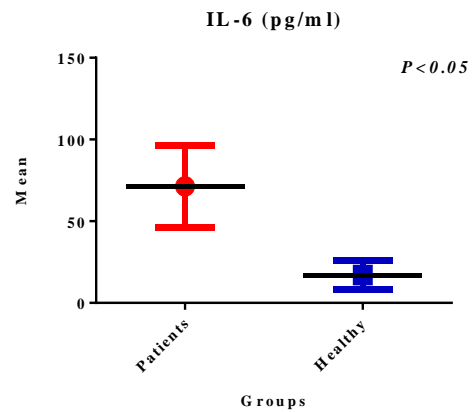
		Count	Percent	P value
Age groups (groups)	1-20	13	43.3%	P<0.05*
	21-40	7	23.3%	
	41-60	7	23.3%	
	>60	3	10.0%	
Gender	Females	18	60.0%	P>0.05
	Males	12	40.0%	

**2. Comparative mean levels of IL-6 between T1DM patients versus healthy**

Results of present study showed increased levels of IL-6 in T1DM patients (71.36±25.09) than healthy (17.11±8.84) with significant different ( $p < 0.05$ ) (table 2 and figure 1).

**Table 2: Comparative Mean Levels of IL-6 Between T1DM Patients Versus Healthy.**

Groups	N	Mean	SD	p value	
IL-6	Patients	30	71.36	25.09	P<0.001***
	Healthy	20	17.11	8.84	



**Figure 1: Comparative mean levels of IL-6 between T1DM patients versus healthy.**

**3. Comparative positivity of CRP between T1DM patients versus healthy**

Results of table (3) showed the positivity of CRP was 100% (negativity 0%) in T1DM patients than positivity of healthy that scored 0% (negativity 100%) with significant different ( $p < 0.05$ ).

**Table 3; Comparative Positivity of CRP Between T1DM Patients Versus Healthy.**

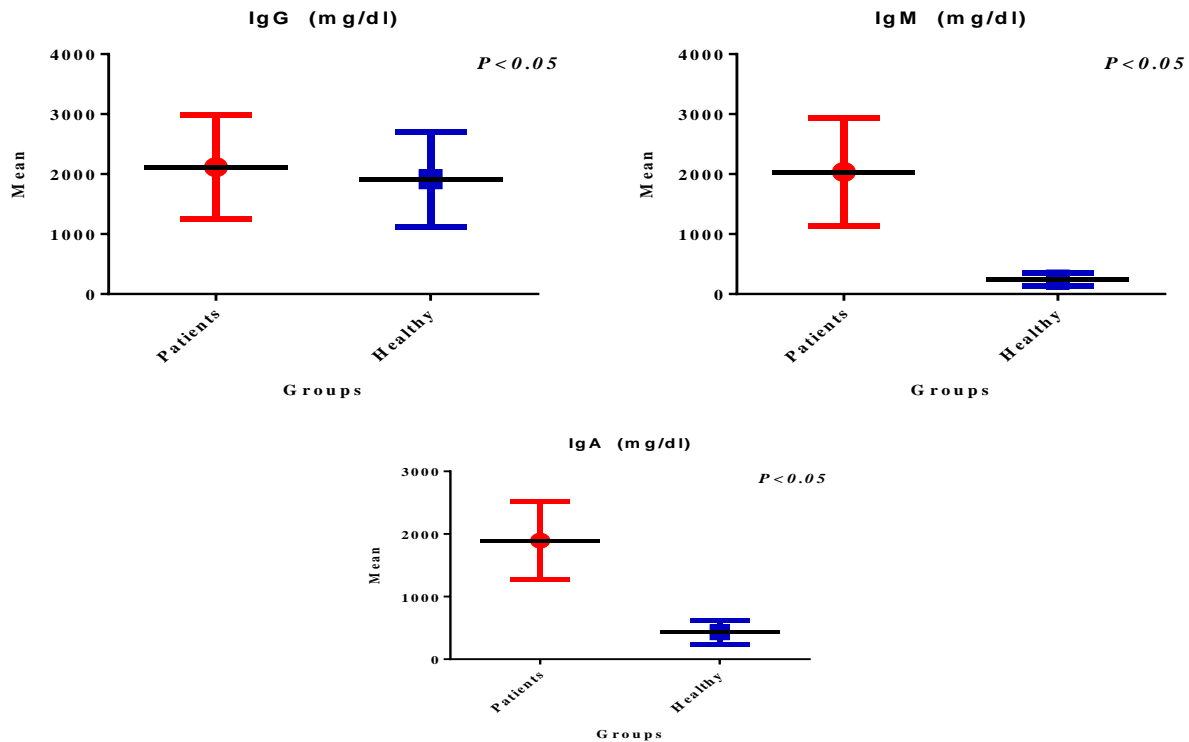
		Groups		Total
		Patients	Healthy	
CRP	Positive	n 30	0	30
	%	100.0%	0.0%	60.0%
Negative	n	0	20	20
	%	0.0%	100.0%	40.0%
Total	n	30	20	50
	%	100.0%	100.0%	100.0%
p value		p<0.001***		

**4. Comparative Mean Levels of immunoglobulin between t1dm Patients Versus Healthy**

Results of present study showed raised levels of IgG, IgM, IgA in T1DM patients (2116.22±871.70, 2035.27±901.18, 1894.13 ±622.33) than healthy (1916.35±791.96, 242.50±103.01, 432.14±189.82) with significant different ( $p < 0.05$ ) (table 4 and figure 2).

**Table 4; comparative mean levels of immunoglobulin between T1DM patients versus healthy.**

Immunoglobulin		N	Mean	SD	P value
IgG	Patients	30	2116.22	871.70	P<0.05*
	Healthy	20	1916.35	791.96	
IgM	Patients	30	2035.27	901.18	P<0.001***
	Healthy	20	242.50	103.01	
IgA	Patients	30	1894.13	622.33	P<0.001***
	Healthy	20	432.14	189.82	

**Figure 2: Comparative Mean Levels Of Immunoglobulin Between T1dm Patients Versus Healthy.****DISCUSSION**

Absolute insulin insufficiency results from the chronic autoimmune illness known as type 1 diabetes mellitus (T1DM), which destroys the pancreatic cells that produce insulin. (Besser et al., 2022). Unlike type 2 diabetes mellitus (T2DM), which is linked to a relative insulin deficit, type 1 diabetes (T1DM) often develops in childhood and adolescence rather than in maturity. (Buchmann et al., 2023). Owing to the grave health implications, type 1 diabetes can shorten life expectancy and lower quality of life. (Bekele et al., 2022). T1DM affects children and adolescents and represents approximately 5-10% of diabetes cases (Al-Hasso., 2023); (Pasi and Ravi, 2022). T1D is one of the most common autoimmune diseases in young people (Hallström et al., 2021); (Felton et al., 2023). Data on health care for patients with T disease, for both sexes, showed that care is worse in young children with T disease, especially during adolescence, and that focusing on the cause of these differences and their treatment may give greater chances of achieving better results (de Vries et al., 2023). The highest incidence of T1DM is for the age group in childhood and adolescence (Jammal, 2023). While the statistical study conducted by Amadi et al

(2020), for the five years (2014-2018) in Nigeria showed that the highest infection rate was for adults aged (30-39) years, with significant differences with a statistically significant p value (<0.001). It was found that the most common age group in Egyptian patients with T1DM is adults, higher than in other population groups. Moreover, data show a high prevalence of the disease among both sexes (Abouali et al, 2020); (Rodrigues et al., 2023). The rates of infection with T1DM in children aged (0-14) years do not show any differences between the sexes. As for people aged between (15-39) years, it was found that the infection rate in males reaches double, as for those aged 40 years and above is uncertain, but it shows fairly equal rates between males and females. The reason for the higher incidence in males is the hormonal effect and higher peripheral insulin resistance (Wandell and Carlsson, 2013). Around the world T1DM is becoming more prevalent among pediatrics. The most common cause of mortality in children is diabetic ketoacidosis (DKA). It affects up to one-third of people with T1DM (Baroum and. Ahmed, 2023). Diabetes mellitus is one of the largest global health concerns of recent times. Women with diabetes mellitus have a higher excess risk of all-cause mortality and more vascular events than

men, Focusing on T1DM, this could be caused by gender inequalities in delivered diabetes care (Bak et al., 2023).

Males are more susceptible to developing T1DM compared to females, and it appears that females have a higher sensitivity to insulin, which affects glucose levels. The reason for the difference between the sexes is explained by the fact that affected females have many factors that affect glucose levels, such as hormones that Stimulates ovulation/menstrual cycle and associated change in the time of menopause (Tatti and Pavandeeep, 2022). Accordingly, treatment with male hormones appears to increase insulin resistance in women, and this hormonal effect may explain the lower insulin sensitivity in men. In recent years, T1DM has become one of the biggest global health concerns, as women with the disease are considered more likely to die than men. T1DM is the only autoimmune disease that predominantly affects adult males rather than older females, distinguishing it from all other forms of autoimmunity (Tonolo, 2023). In Asia, the mortality rate associated with T1DM due to heart and kidney disease was 2-3 times higher among females compared to males, and this may be due to the disparity between the sexes in the quality of care and health habits (Lim et al., 2022). The use of daily insulin doses continuously increased in females by 54%, compared to males by 47% (Boettcher et al., 2021). The field study conducted by Amadi et al (2020), showed that the total prevalence of T1DM between 2014 and 2018, fluctuated between males and females, as females showed the highest incidence rate (annual prevalence) compared to males during the five-year period. The reason for this is the result of urban-to-rural migration, environmental factors and other unidentified social and cultural factors among the population.

A study Kalaivani et al. (2023), showed that there is an increase in IL-6 levels in the serum of patients with T1DM, who suffer from poor blood sugar control compared to the non-diabetic group, therefore diabetes could play a major role in the inflammatory response. A study Hammed et al (2012) found that the average level of IL-6 in serum in children with T1DM was significantly higher compared to healthy people ( $P \geq 0.0001$ ), this is due to the presence of a chronic inflammatory condition in affected children. Which in turn is reflected in the level of serum IL-6. Which may play a major role in the early stages of atherosclerosis and the development of microvascular complications (Myśliwska al., 2009). A study was conducted on 30 patients with T1DM, aged from (17 - 40) years, and it was found that there was a significant significant increase in the value of CRP and IL-6,  $P$  value =  $[(0.001), (0.001)]$ , respectively, compared to the control group (Mohamed et al., 2020);(Kato et al., 2019).

The results of a study Colhoun et al (2002), which was conducted on 196 people with T1DM and 195 people without diabetes, aged between 30-55 years, showed that

the CRP level was high in females with diabetes compared to those without diabetes, with a significant increase ( $P$  value = 0.001). While the CRP value was similar between diabetic and non-diabetic males, this may reflect special sensitivity to insulin levels or insulin resistance. Elevated CRP concentrations, which remain within the normal range, have been associated with an increased incidence of coronary heart disease in non-diabetic people, compared to elevated levels in people with T1DM (Kilpatrick et al., 2000). High levels of hsCRP (High-sensitivity CRP) observed in children with T1DM compared to healthy controls, with a similar body mass index (BMI), indicate a basal inflammatory state that can increase the risk of cardiovascular disease (Pérez-Segura et al., 2020). A study (King et al., 2003) showed that there is an increase in CRP concentrations with increasing HbA1c levels ( $\geq 9.0\%$ ), as these results indicate a relationship between blood sugar control and systemic inflammation in people with diabetes.

There appears to be a correlation between T1DM infection and an increase in the plasma percentage and structure of IgG, a decrease in monogalactose, and an increase in immunoglobulin binding. Thus, an increase in the number of autoantibodies is an important indicator of the development of T1DM (Rudman et al., 2022). It was found that there was a decrease in IgG production in the second week of T1DM compared to healthy controls (Otsubo et al., 2023). It was observed that there was a significant increase in the concentration of IgG and IgM antibodies in the serum of people with T1DM at a younger age (Fayed et al., 2014). The normal immune system is capable of regulating autoimmunity through multiple mechanisms, but in the case of T1DM, normal IgM is unable to perform its function in immune regulation (Wilson et al., 2022). The study conducted by (Abouali et al., 2020) for patients with T1DM showed that there is a decrease in the level of IgA [Selective IgA deficiency (SIgAD)]. Uncertainty surrounds the function of elevated serum IgA in the etiology of T1DM and other autoimmune illnesses that have been linked to it. According to the study, children and teenagers with elevated serum IgA exhibit distinct clinical, laboratory, and demographic characteristics from those with normal IgA levels. IgA in serum (Thakkar et al., 2022).

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