Egypt. Acad. J. Biolog. Sci., 12(2):41-56 (2020)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg

Immunological Profile of Type I Diabetes in Some Egyptian Patients

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ARTICLE INFO

Article History

Received: 4/6 /2020

Accepted: 2/ 8/2020

Keywords:

Diabetes mellitus, immunological parameters, Egypt.

ABSTRACT

Background: Diabetes mellitus is regarded to be one of the five leading induces of death in the world. **Aim:** The aim of this study is to estimate changes in the levels of biochemical parameters as fasting blood sugar (FBS), postprandial blood sugar (PP2BS) and glycosylated hemoglobin (HbA1c) together with immunological parameters as Complement proteins (C3 and C4), specific Immunoglobulin's (IgA, IgE, IgG, IgM) and C-peptide of type 1 diabetic patients to obtain a full immunological and physiological profile for type 1 diabetes. **Subjects and Methods:** Blood samples from 25 type 1 diabetic patients and 5 healthy control subjects were randomly selected from Zagazig University hospitals in Sharqiyya, Egypt. Serum blood sugar and glycosylated hemoglobin were measured following standard protocols and by using a chemical analyzer. C3, C4, IgA, IgE, IgG, IgM, and C-peptide were assayed by ELISA. **Results:** FBS, PP2BS and HbA1c significantly increased in type 1 diabetic patients compared to healthy control subjects. C4 and C-peptide significantly decreased in type 1 diabetic patients compared to healthy control subjects. IgG and IgM showed a significant increase in type 1 diabetic patients compared to healthy control subjects. **Conclusion:** Type 1 diabetes in the current studied subjects could be described as characterized by classically active complement system (abnormal C4 levels) which is induced by increased levels of IgG and IgM together with increased blood sugar levels (FBS, PP2BS and HbA1c) and decreased levels of C-peptide in serum.

INTRODUCTION

Diabetes is reaching epidemic proportions worldwide; if it continues increasing at the current rate, diabetes will affect almost 10% of the world population by the year 2035 (Ghosh *et al.,* 2015).Diabetes mellitus is a chronic disease that characterized by hyperglycemia which is due to deficiency of insulin effect, and results in abnormal metabolism of carbohydrate, protein, and fat and may lead to any complications including heart, kidney, eye and nerve diseases (Goldstein, 2002; Sperling and Jenson, 2000).

There are two main types of diabetes, type I insulin-dependent diabetes mellitus (IDDM), which is usually manifested in childhood or adolescence, and characterized by pancreatic β-cell destruction mediated by immune mechanisms, whereas type II non-insulin dependent diabetes mellitus (NIDDM), is characterized by insulin resistance, with associated with a defect in insulin secretion, it occurs after age 40, but it may occur at any age, other types of diabetes may be secondary due to other diseases (Barnett and Braunstein, 2001).

In nondiabetic individuals, fasting plasma glucose concentrations (i.e., following an overnight 8- to 10-h fast) generally range from 70 to 110 mg/dl (American Diabetes Association, 2001). Post-prandial hyperglycemia is defined as a plasma glucose level exceeding 140 mg/dl (Pratley and Weyer, 2001).

The diagnosis of diabetes should be made with an HbA1c level of ≥ 6.5%. Diagnosis should be confirmed with a repeat HbA1c test unless clinical symptoms and high glucose levels >200 mg/dl are present (American Diabetes Association, 2004).

The complement system is an effector of both adaptive and innate immunity. It is composed of more than 30 plasma and cell-membrane proteins that are synthesized by hepatocytes or locally in peripheral tissues and that normally circulate as inactive precursors (proproteins). Complement proteins interact with one another in three enzymatic activation cascades known as the classical, the alternative, and the mannose-binding lectin (MBL) pathways (Walport, 2001).

Complement C3 is the central component of the human complement system. It is consisting of an α-chain and a β-chain that are connected by cysteine bridges. C3 in its native form is inactive. Cleavage of C3 into C3b and C3a is a crucial step in the complement activation cascade, which can be initiated by one or more of the three distinct pathways, called alternative, classical and lectin complement pathways (Ashok *et al.,* 2012). C3 functions include opsonization and phagocytosis of pathogens/apoptotic cells, clearance of immune complexes, and chemotaxis (Trouw *et al.* 2008 and Lutz, 2012).

Complement component C4 plays a central role in classical and lectin pathways of complement. There are two isotypic forms of C4, C4A, and C4B that differ in their chemical and serological properties. C4 deficiencies are often seen in association with infection diseases (Jaatinen *et al.,* 2002).

Antibodies are gamma globulin proteins that are found in blood or other body fluids and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses (Janeway *et al.,* 2005).

There are five antibody isotypes known as an immunoglobulin (IgA, IgD, IgE, IgG, and IgM) that have different roles in the immune system (Alberts *et al*., 2002). There are five types of mammalian Ig heavy chain denoted by the Greek letters: α (alpha), δ (delta), ε (epsilon), γ (gamma), and μ (mu) (Janeway, 2001). The type of heavy chain present defines the class of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively (Rhoades and Pflanzer, 2002).C-peptide is produced in equal amounts to insulin and can, therefore, be used to assess endogenous insulin secretion, including in patients who are insulin-treated. Assessment of insulin secretion is potentially helpful in clinical practice: differences in glycaemic treatment requirements between Type 1 and Type 2 diabetes mainly relate to the development of absolute insulin deficiency in the former (Wahren *et al.,* 2012).

C-peptide persistence has been associated with improved continuous glucose monitoring (CGM) ‘time in range’ in a large paediatric cohort of people with recently diagnosed type 1 diabetes (Buckingham *et al.,* 2015) and with lower glucose variability and low-glucose events in type 2 diabetes (Hope *et al.,* 2018).

The aim of this study is to estimate changes in the levels of FBS, PP2BS and HbA1c together with immunological parameters as C3 and C4, IgA, IgE, IgG, IgM and C-peptide of type 1 diabetic patients to obtain a full immunological and physiological profile for type 1 diabetes in the studied subjects.

MATERAILS AND METHODS

Patients:

Blood samples from 25 type 1 diabetic patients and 5 healthy control subjects were randomly selected from Zagazig University

hospitals in Sharqiyya, Egypt. Ethical approval has been issued to the current project by the official ethical committee, faculty of Science, Al-Azhar University, Egypt. Consent was taken from all patients before blood sampling. Patients were grouped, depending on the clinical examination that has done by hospitals, for diabetes mellitus as followed:

Group I: Twenty-five patients with type 1 diabetes or insulin-dependent diabetes mellitus (IDDM).

Group II: Five healthy subjects.

Blood Collection:

The collections of samples were taken from March, 2017 until March, 2018. All samples were taken from patients that have fasted overnight and other samples were taken after 2hr postprandial. A sample of blood consisting of 5ml was obtained from the standard radial vein by a sterile disposable syringe from each patient at the hospitals.

A part of the samples was collected on sodium fluoride for fasting and postprandial blood sugar. Another part of the samples was collected on EDTA (ethylene diamine tetra acetic acid) for glycosylated hemoglobin (HbA1c). The third part of the samples was poured into a clean test tube without anticoagulant and left for 2-3 minutes in a water bath (37°C), then centrifuged at 3000 rpm for 6-10 minutes. The serum was separated and transferred to label multiple clean eppendrof tubes with patient full information then stored at -20°C for various immunological analyses. The Frozen samples were transferred to Al-Azhar University hospitals.

Methods:

Biochemical Parameters:

Serum blood glucose and Glycosylated hemoglobin (HbA1c) were determined according to the method described by Tietz (1995) and Trivelli *et al.* (1971) respectively using available kits of spectrum, Egypt by Chem 7 (chemical analyzer).

Immunological Parameters:

Serum complement C3 and C4 concentration were determined by turbidimetric assay (ELISA) according to the method described by Lachmann *et al.* (1973) using available kits of DIALAB, Austria by Beckman Coulter (ELISA system).

Serum immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) concentration were determined by turbidimetric assay (ELISA) according to the method described by Friedman and young (2001), Price *et al.* (1983) and young (2000) respectively using available kits of BioSystems, Switzerland by Beckman Coulter.

Serum immunoglobulin E (IgE) and serum C-peptide concentration were determined by enzyme immunoassay (ELISA) according to the method described by Michel *et al.* (1980) and Bonger and Garcia-webb (1984) respectively using available kits of BioCheck, Inc, America for IgE and bioactive diagnostic, Germany for C-peptide, by Beckman Coulter.

Statistical Analysis:

The statistical package for the social sciences SPSS/PC computer program (version 20) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA). Data were expressed as mean ± S.E. and P values less than 0.05 were considered significant.

RESULTS

Blood samples from randomly selected 25 type 1 diabetic patient have been collected, 13 (52%) of these patients were males where the other 12 (48%) were females, their ages ranged between 4 to 19 years (mean age, 14.08±0.75 years). Samples from the other 5 healthy control subjects have also been collected, 3 (60%) of these subjects were males where the other 2 (40%) were females, their ages ranged between 4.5 to 18 years (mean age, 13.6±2.25 years). from the hospital were examined (Fig. 1).

The results of the current study were focused on two parts. The first part was biochemical parameters such as fasting blood sugar (FBS), postprandial blood sugar (PP2BS), and glycosylated hemoglobin

(HbA1c) of type 1 diabetic patients. The second part was Immunological parameters such as C3, C4, IgA, IgE, IgG, IgM, and C-peptide of type 1 diabetic patients.

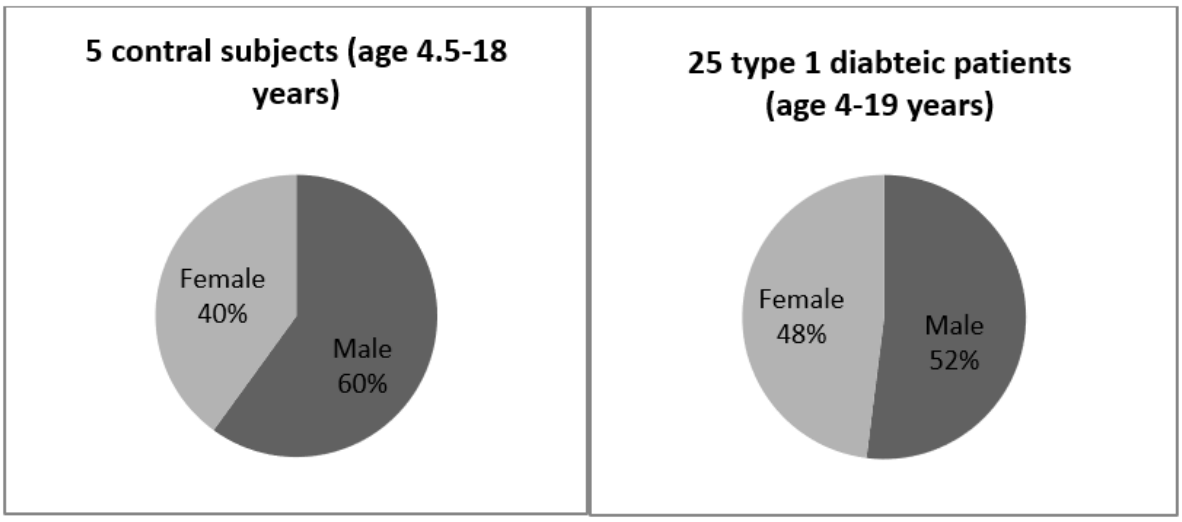

Fig.1: Study groups for type 1 diabetes

Biochemical Parameters:

Fasting Blood Sugar (FBS):

The current results showed a highly significant increase of FBS (at P<0.01) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 2, where mean value and S.E of FBS were (183.8±14.08) mg/dl in type 1 diabetic patients and were (78.4±1.63) mg/dl in healthy control subjects. The normal range of fasting blood sugar in human plasma is 70-110mg/dl.

Postprandial Blood Sugar (PP2BS):

The current work also showed a very highly significant increase (at P<0.001) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 2, where the mean value of PP2BS and S.E were (247.32±16.96) mg/dl in type 1 diabetic patients and were (96.8±1.53) mg/dl in healthy control subjects. The normal range of postprandial blood sugar in human plasma is up to 140mg/dl.

Table 1: The means ± SE of FBS, PP2BS, HbA1c, C3, C4, IgA, IgE, IgG, IgM and C-peptide in type 1 diabetic patients and control subjects

parameters / Groups		FBS 70-110 mg/dl	PP2BS Up to 140 mg/dl	HbA1c 4-6 %	C3 80-160 mg/dl	C4 20-40 mg/dl	IgA (mg/dl)	IgE 0-100 IU/ml	IgG (mg/dl)	IgM (mg/dl)	C-peptide 0.5-3 ng/ml
Diabetic group	Means ± SE	183.8 ± 14.08	247.32 ± 16.96	10.08 ± 0.46	127.5 ± 7.38	24.16 ± 2.77	159.92 ± 13	61.66 ± 10.29	1422.29 ± 72.79	209.81 ± 17.38	0.33 ± 0.01
Control group	Means ± SE	78.4 ± 1.63	96.8 ± 1.53	4.32 ± 0.05	108.4 ± 3.53	37.3 ± 0.68	104.48 ± 10.55	27.02 ± 7.63	883.46 ± 52.79	81.06 ± 8.04	1.84 ± 0.2
F-value		10.8	15.3	29.9	1.29	4.35	3.46	2.16	10.47	10.58	261.5
Probability		**	***	***	N.S	*	N.S	N.S	**	**	***

The normal range of IgA in children at age 4-6 years old is 27-195 mg/dl, 7-9 years old is 34-305 mg/dl, 10-11years old is 53-204 mg/dl, 12-13 years old is 58-358 mg/dl, 14-15 years old is 47-249 mg/dl and 16-19 years old is 61-348 mg/dl.

The normal range of IgG in children at age 4-6 years old is 504-1464 mg/dl, 7-9 years old is 572-1474 mg/dl, 10-11 years old is 698-1560 mg/dl, 12-13 years old is 759-1549 mg/dl, 14-15 years old is 716-1711 mg/dl and 16-19 years old is 549-1584 mg/dl.

The normal range of IgM in children at age 4-6 years old is 24-210 mg/dl, 7-9 years old is 31-239 mg/dl, 10-11 years old is 31-179 mg/dl, 12-13 years old is 35-239 mg/dl, 14-15 years old is 15-188 mg/dl and 16-19 years old is 23-259 mg/dl.

Significantly different at (P<0.05)

N.S =non-significant (p<0.05) =* (p<0.01) =** (p<0.001) =***

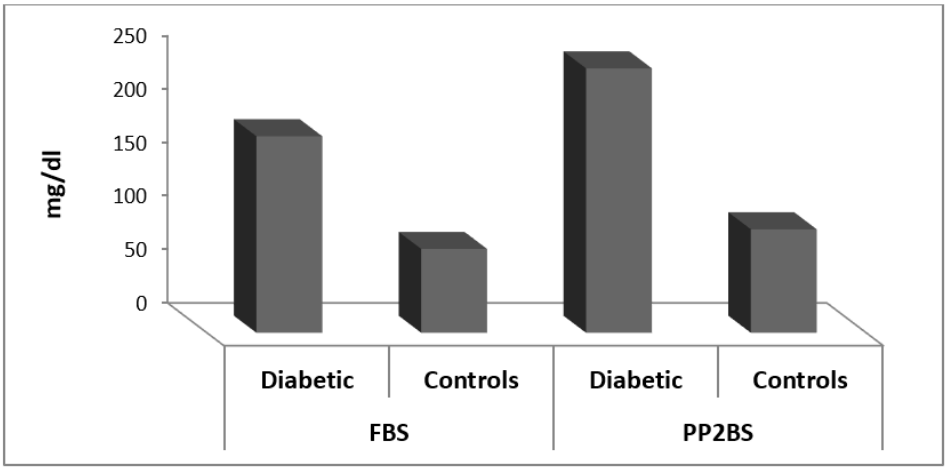

Fig. 2: The means of FBS and PP2BS in type 1 diabetic patients and control subjects

Glycosylated hemoglobin (HbA1c):

It is expressed in a very highly significant increased level (at P<0.001) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 3, where mean value and S.E for HbA1c were (10.08±0.46 %) in type 1diabetic patients and were (4.32±0.05 %) in healthy control subjects. The normal range of HbA1c in humans is 4-6% (good control 4.5-7%, fair control 7.1-8.4%, and uncontrolled >8.5).

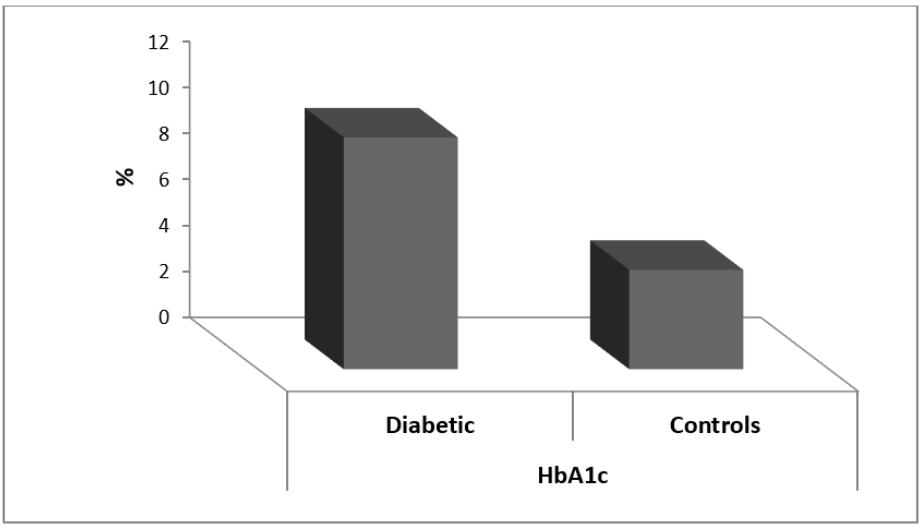

Fig. 3: The means of HbA1c in type 1 diabetic patients and control subjects

Immunological Parameters:

Levels of Serum Complement C3:

The current results showed no statistically significant differences (at P<0.05) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 4, where the mean value of C3 and S.E were (127.5±7.38) mg/dl in type 1 diabetic patients and were (108.4±3.53) mg/dl in healthy control subjects. The normal range of C3 in human serum is 80 – 160 mg/dl.

Out of 25 patients, 22 cases represent (88%) had normal values of C3 while 3 cases represent (12%) had abnormal levels of C3 values as shown in Fig. 10.

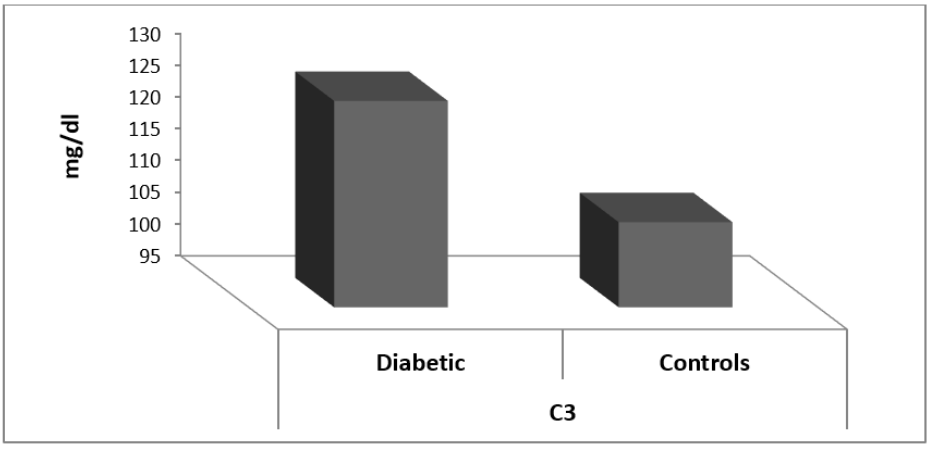

Fig. 4: The means of C3 in type 1 diabetic patients and control subjects

Levels of Serum Complement C4:

The current work showed a significant decrease (at P<0.05) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 5, where mean value and S.E of C4 were (24.16±2.77) mg/dl in type 1 diabetic patients and were (32.5±1.34) mg/dl in healthy control subjects. The normal range of C4 in human serum is 20-40 mg/dl.

Comparison between the high, low and normal levels of C4 has revealed the following: C4 was high in 6 (24%) patients, low in 15 (60%) patients, and normal in 4 (16%) patients type 1 of diabetic patients. The normal range of C4 in human serum is 20-40 mg/dl shown in Fig. 6.

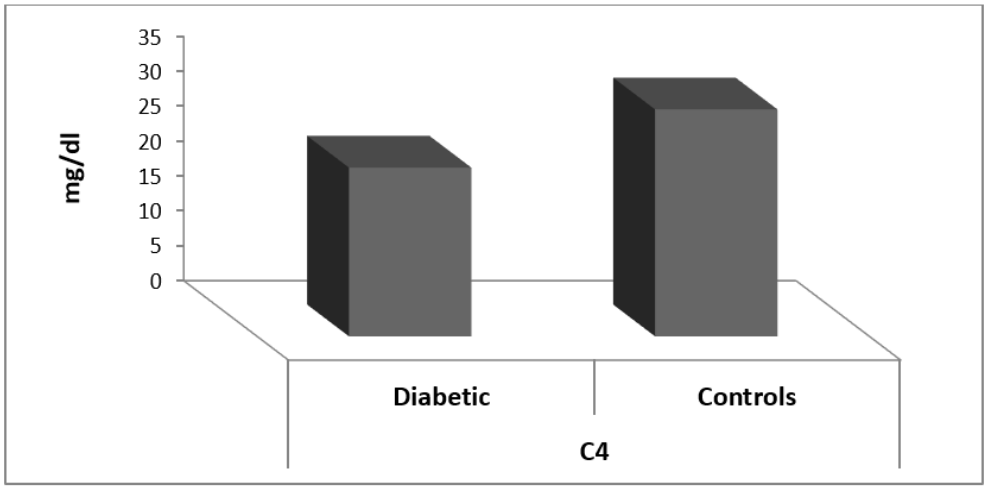

Fig. 5: The means of C4 in type 1 diabetic patients and control subjects

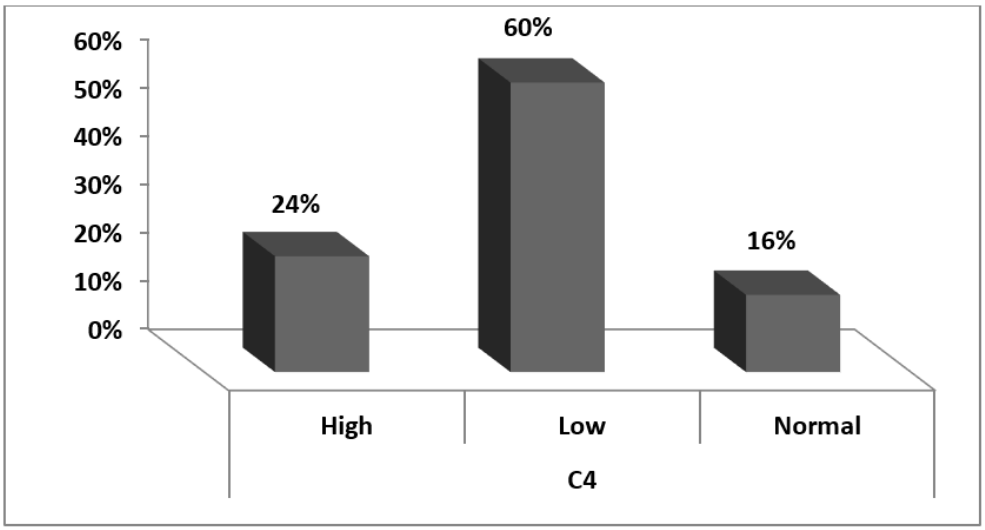

Fig. 6: Comparison between the high, low and normal levels of C4 in type 1 diabetic patients comparing with reference values

Levels of Serum Immunoglobulin A (IgA):

The current results showed that, no statistically significant differences (at $P<0.05$) in type 1diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 7, where the mean value of IgA and S.E were (159.92±13) mg/dl in type 1 diabetic patients of diabetes mellitus and were (104.48±10.55) mg/dl in healthy control subjects. The normal range of IgA in children at age 4-6 years old is 27-195 mg/dl, 7-9 years old is 34-305 mg/dl, 10-11years old is 53-204 mg/dl, 12-13 years old is 58-358 mg/dl, 14-15 years old is 47-249 mg/dl and 16-19 years old is 61-348 mg/dl. All of 25 cases represent (100%) had normal values of IgA as shown in Fig. 10.

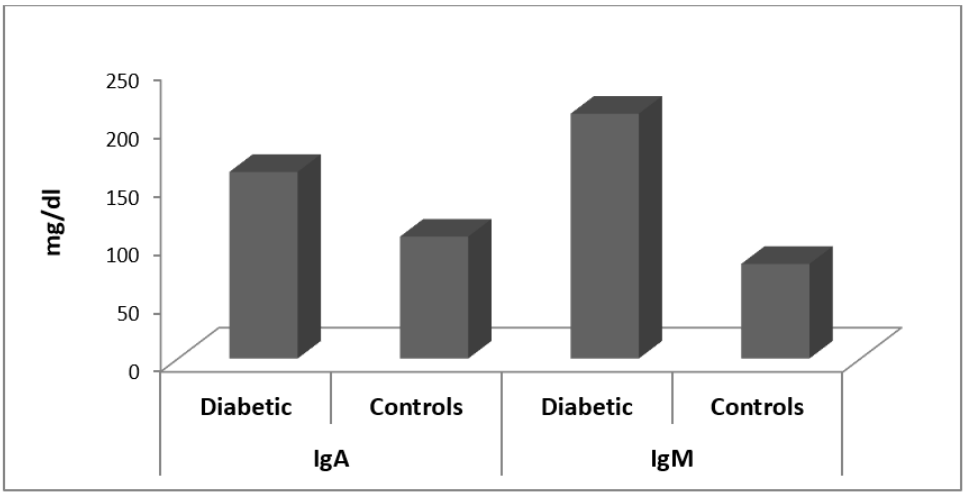

Fig. 7: The means of serum IgA and IgM in type 1 diabetic patients and control subjects

Levels of Serum Immunoglobulin E (IgE):

The current work showed that, no statistically significant differences (at P<0.05) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 8, where mean value and S.E of IgE were (61.66±10.29) IU/ml in type 1 diabetic patients and were (27.02±7.63) IU/ml in healthy control subjects. The normal range of IgE in human serum is 0-100 IU/ml.

Out of 25 patients, 20 cases represent (80%) had normal values of IgE while 5 cases represent (20%) had abnormal levels of IgE values as shown in Fig. 10.

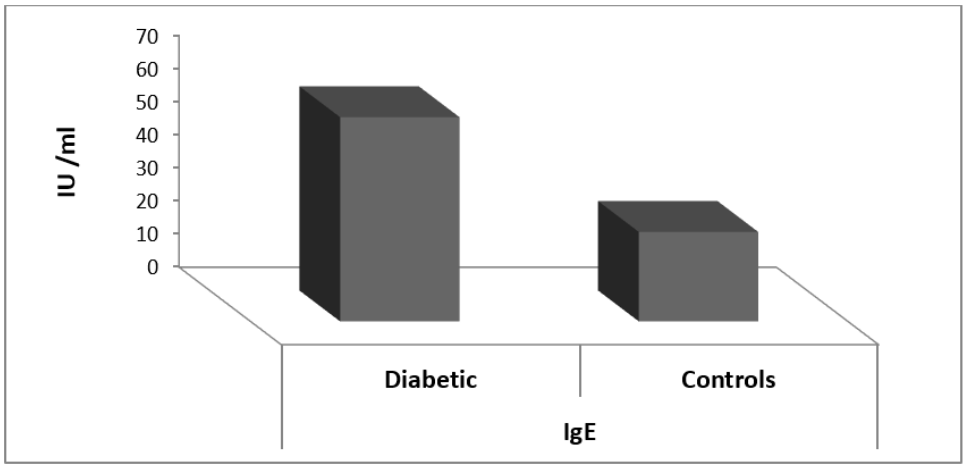

Fig. 8: The means of serum IgE in type 1 diabetic patients and control subjects

Levels of Serum Immunoglobulin G (IgG):

The current results showed a highly significant increase (at P<0.01) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 9, where the mean value of IgG and S.E were (1422.29±72.79) mg/dl in type 1 diabetic patients and were (883.46±52.79) mg/dl in healthy control subjects. The normal range of IgG in children at age 4-6 years old is 504-1464 mg/dl, 7-9 years old is 572-1474 mg/dl, 10-11 years old is 698-1560 mg/dl, 12-13 years old is 759-1549 mg/dl, 14-15 years old is 716-1711 mg/dl and 16-19 years old is 549-1584 mg/dl.

Out of 25 patients, 13 cases represent (52%) had normal values of IgG while 12 cases represent (48%) had abnormal levels of IgG values as shown in Fig. 10.

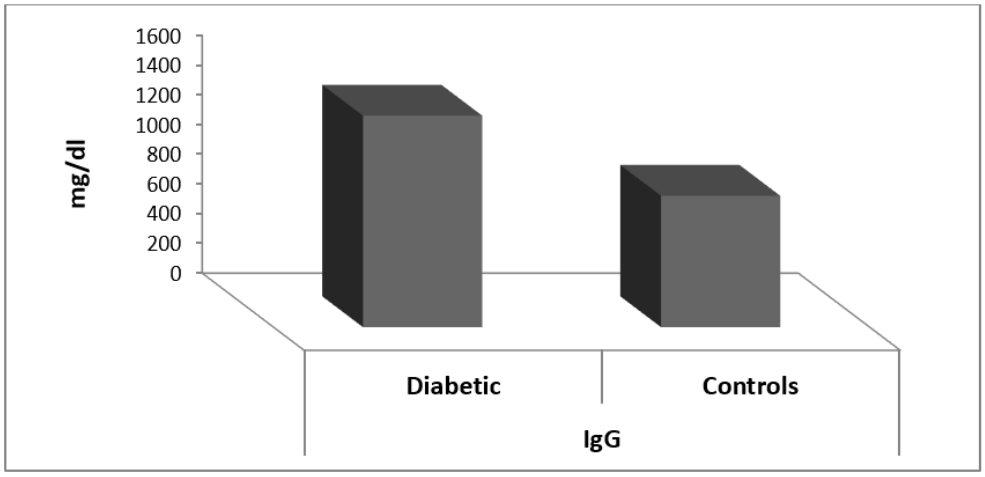

Fig. 9: The means of serum IgG in type 1 diabetic patients and control subjects

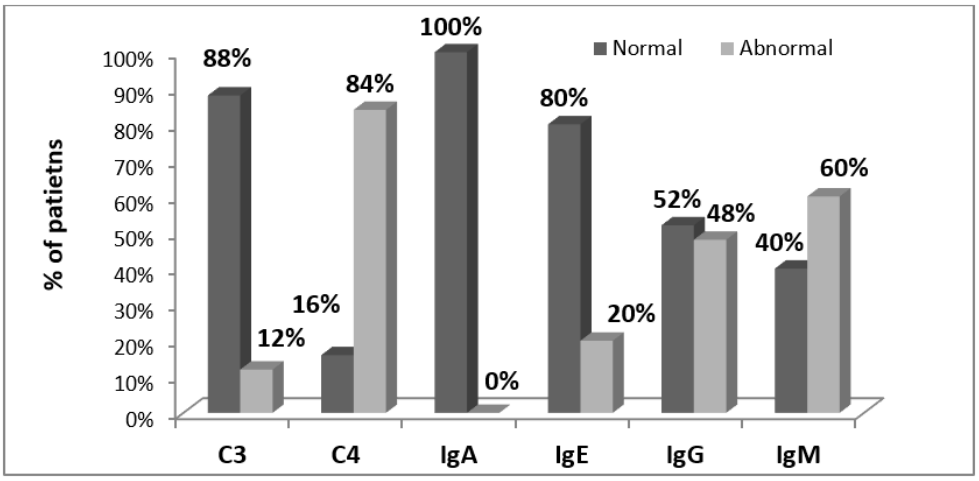

Fig. 10: Normal and abnormal levels of C3, C4, IgA, IgE, IgG, and IgM in type 1 diabetic patients

Levels of Serum Immunoglobulin M (IgM):

The current work showed a highly significant increase (at $P<0.01$) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 7 where mean value and S.E of IgM were (209.81±17.38) mg/dl in type 1 diabetic patients and were (81.06±8.04) mg/dl in healthy control subjects. The normal range of IgM in children at age 4-6 years old is 24-210 mg/dl, 7-9 years old is 31-239 mg/dl, 10-11 years old is 31-179 mg/dl, 12-13 years old is 35-239 mg/dl, 14-15 years old is 15-188 mg/dl and 16-19 years old is 23-259 mg/dl.

Out of 25 patients, 10 cases represent (40%) had normal values of IgM while 15 cases represent (60%) had abnormal levels of IgM values as shown in Fig. 10.

Levels of Serum C-peptide:

The current results showed a very highly significant decrease (at P<0.001) in type 1 diabetic patients when compared with healthy control subjects as in Table 1 & Fig. 11, where the mean value of C- peptide, and S.E were (0.33±0.01) ng/ml in type 1 diabetic patients and was (1.84±0.2) ng/ml in healthy control subjects. The normal range of serum C-peptide in human serum is 0.5-3 ng/ml.

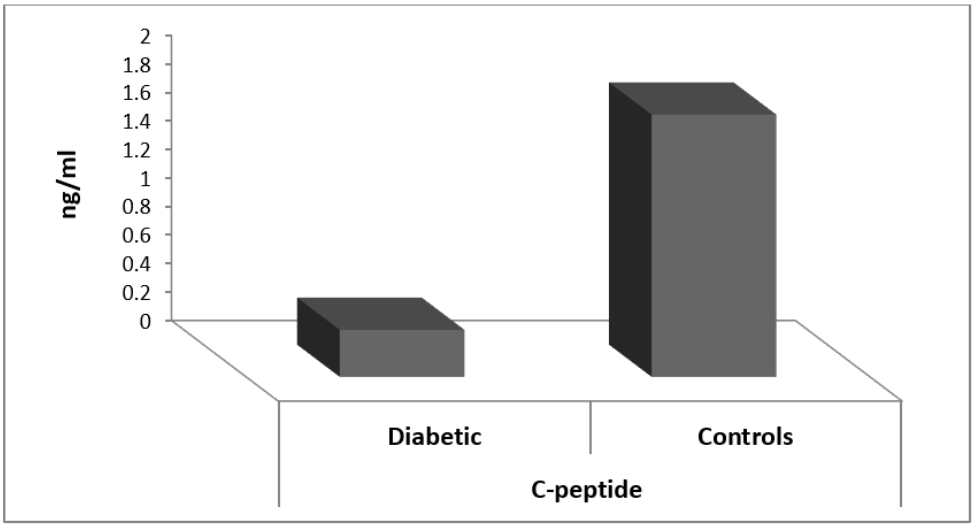

Fig. 11: The means of serum C-peptide in type 1 diabetic patients and control subjects

DISCUSSION

Fasting blood sugar, postprandial blood sugar and glycosylated hemoglobin (HbA1c):

The obtained results revealed that fasting blood glucose showed a highly significant increase (at P<0.01), postprandial blood sugar showed a very highly significant increase (at P<0.001) and glycosylated hemoglobin (HbA1c) showed a very highly significant increase (at P<0.001) in type 1diabetic patients when compared with healthy control subjects. This may be due to the absolute loss of production of insulin by the pancreatic islets of Langerhans.

The results also, validate well with the findings of many authors. Patel *et al.* (2016) showed that diabetic subjects had a higher value of FBS, PP2BS, and HbA1c than non-diabetic subjects. Khan *et al.* (2015) found that patients with HbA1c >6.5% had significantly higher levels of fasting blood sugar (FBS). Alghobashy *et al.* (2013) recorded that diabetic children had significantly higher white blood cell count, HBA1C than healthy children. Tankova *et al.* (2012) suggested that HbA1c ≥ 6.1% combines high specificity and sensitivity and may be used for diagnosing diabetes. Cavagnolli *et al.* (2011) reported that HbA1c ≥ 6.5% (48mmol /mol) showed limited sensitivity to diabetes diagnosis, although with high specificity. Beck (2011) reported that the level of HbA1c was elevated in type 1 diabetes. Bao *et al.* (2010) studied that an HbA1c threshold of 6.3% was highly specific for detecting undiagnosed diabetes. This optimal HbA1c threshold may be suitable as a diagnostic criterion for diabetes. Kim *et al.* (2008) exhibited that measurement of FPG and HbA1c might be a more sensitive screening tool for identifying high-risk individuals with diabetes at an early stage.

On the other hand, these results are in disagreement with Svendsen *et al.* (1982) who found that type 1 (insulin-dependent) diabetic patients were with a constant hemoglobin A1C during the preceding 2 years.

Complement proteins C3 and C4:

The obtained results revealed that complement proteins C3 showed no statistically significant differences (at P<0.05) and complement proteins C4 showed a significant decrease (at P<0.05) in type 1 diabetic patients when compared with healthy control subjects. The decrease in the levels of complement proteins C4 indicated activation of the complement system in type 1 diabetic patients. The decrease levels of C4 indicate the classical pathway activation of the complement system when C3 is either normal or abnormal.

The results were in accordance with the findings of several authors. Bus *et al.* (2018) found that C4d deposits were more prevalent in cases with diabetic nephropathy (DN) than in cases without DN in both the glomeruli and the arterioles. Complement activation is correlated with both the presence and severity of DN, suggesting that the complement system is involved in the development of renal pathology in patients with diabetes and is a promising target for inhibiting and/or preventing DN in these patients. Besides, Singh *et al.* (2016) reported that the mean value of serum C4 was significantly reduced in T1DM. C3 in DM patients was within a normal range. They conclude that C4 deficiency was present in T1DM and it was related to lower age. Barnett *et al.* (1984) showed that a low plasma C4 concentration is significantly associated with diabetic microangiopathy and might be a predictor of a subgroup of insulin-dependent diabetics who have a particular propensity to develop the severe microvascular disease. Also, Vergani *et al.* (1983) recorded that low C4 values were seen in insulin-dependent diabetes irrespective of the duration of the disease and did not appear to correlate with complement activation.

These findings results were nearly similar to those of Blackwell *et al.* (1988) and Charlesworth *et al.* (1987) who found that lower levels of C3 and C4 components of the complement system have been reported for patients with insulin-dependent diabetes mellitus (IDDM).

These results were in disagreement with the results of Hussein and Abdul-Adhim (2006) who showed that a significant increase (p<0.05) in serum C3 while there is no significant difference (p>0.05) in the concentration of the complement C4. Besides, Vialettes *et al.* (1983) reported that a significant decrease in C3 and a mild increase of C3A was found in the recent-onset diabetes group.

Immunoglobulin's (Igs):

The obtained results indicated that immunoglobulin G (IgG) and immunoglobulin M (IgM) showed a highly significant increase (at P<0.01) in type 1 diabetic patients when compared with healthy control subjects. This may be due to an increase in both blood sugar levels and the duration of disease.

The results also were in accordance with Svensson *et al.* (2012) who reported that an increase in IgG levels by age indicates that adult levels are reached later than in previously studied cohorts, thereby indicating a slower maturation of the immune system. Besides, Gorus *et al.* (1998) suggested that IgM concentrations were higher than in control subjects. The changes in total Ig concentrations at onset were largely reversed under insulin therapy. They may reflect exposure to environmental triggers, such as viral infections, or to (relative) insulinopenia prior to clinical disease onset. Whatever their cause, different serum Ig levels exist in different age groups of recent-onset IDDM patients. Moreover, Pishdad and Faghiri (1995) recorded that immunoglobulins G and M that have been reported to be present in statistically significantly higher levels in diabetic patients compared to healthy controls. Also, Pietruska *et al.* (1989) reported that the mean level of IgM and IgG was higher in both types of diabetic patients. Hoddinott *et al.* (1982) showed that IgM levels were higher in females than in males of diabetic patients.

The obtained result was in disagreement with Greco and Maggio (2015) who reported that IgG and IgM levels were not significantly different between diabetic

patients of type 1 and control. AL-Suhaimi *et al.* (2012) and Landgraf *et al.* (2008) suggested that serum immunoglobulins IgG, and IgM of T1DM treated with insulin were found to near normal levels comparable to healthy children. Roio *et al.* (2005) studied the relationship of immunity in pediatric diabetic patients with type 1 diabetes and the susceptibility of these patients to infections through the evaluation of humoral factors. In some patients, no significant difference in plasma IgM levels and lower IgA values and lower IgG levels were detected, and there was an inverse relationship with HbA1c values. They concluded that no strong link between the immunological alterations was found in diabetic patients and the occurrence of infections.

The represented data showed that immunoglobulin A (IgA) and immunoglobulin E (IgE) showed no statistically significant differences (at $P<0.05$) in type 1 diabetic patients when compared with healthy control subjects.

These observations were in agreement with Greco and Maggio (2015) who recorded that no different prevalence rate in IgA deficient patients of type 1 diabetes mellitus. AL-Suhaimi *et al.* (2012) showed that serum immunoglobulins IgA and IgE levels of T1DM treated with insulin were found to near normal levels comparable to healthy patients. Landgraf *et al.* (2008) suggested that serum levels of immunoglobulin A in type 1 diabetes mellitus had normal range. Measurement of serum immunoglobulin A is necessary for all DM-1 particularly before some immunoglobulin A antibody screening.

These results were in disagreement with the results of Giza *et al.* (2016) who reported that serum IgA concentrations differed significantly when grouping patients according to age at entry to the study.

C-peptide:

The present study revealed that C-peptide showed a very highly significant decrease (at $P<0.001$) in type 1 diabetic patients when compared with healthy control subjects may be due to destroying of ß-cells of islets of Langerhans in the pancreas.

Results were in agreement with the findings of Grönberg *et al.* (2020) who reported that a decline of C peptide level in type 1 diabetic patient. Children and adolescents with detectable C peptide after more than 10 years of diabetes duration were predominantly female and had better HbA1c than others during the first 3 years after diagnosis. Shetty *et al.* (2017) showed that a low fasting and low postprandial C-peptide level indicate the benefit from early insulin therapy and the high FC group benefiting from oral hypoglycemic agents. Also, Kuhtreiber *et al.* (2015) found that low C-peptide levels have clinical significance and appear helpful in characterizing groups at-risk for faster C-peptide decline, complications, poorer metabolic control, and severe hypoglycaemia. Low C-peptide levels may be a biomarker for characterizing at-risk patients with type 1 diabetes. Mohammed *et al.* (2013) recorded that C-peptide level in type 1 diabetic patients was very low, while in type 2 and controls was normal. It is better for newly diagnosed diabetic patients to measure their C-peptide levels as a marker for distinguishing between type 1 and type 2 diabetes since some of the type 1 patients are misdiagnosed as type 2. Almeida *et al.* (2013) observed that C-peptide detection was common in type-1 diabetics, particularly shortly after being diagnosed. This result may have clinical implications. Torn *et al.* (2001) found that the median level of C-peptide was slightly lower in the fasting state compared to C-peptide levels in the non-fasting state. C-peptide can be taken either fasting or randomly in patients with type 1 diabetes since the β cell function was stimulated only to a limited extent by food intake. In order to differentiate between autoimmune and non-autoimmune diabetes at diagnosis, C-peptide can preferably be taken randomly during the day since this improves the discrimination.

Conclusion

The present study comes to the conclusion that: Complement proteins C3 showed no statistically significant differences and Complement proteins C4

showed a significant decrease in type 1 diabetic patients when compared with healthy control subjects. Type 1 diabetes in the current studied subjects could be described as characterized by a classically active complement system (abnormal C4 levels) which is induced by increased levels of IgG and IgM together with increased blood sugar levels (FBS, PP2BS, and HbA1c) and decreased levels of C-peptide in serum. C-peptide can be used as a diagnostic tool for differentiation between type 1 and type 2 diabetes.

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