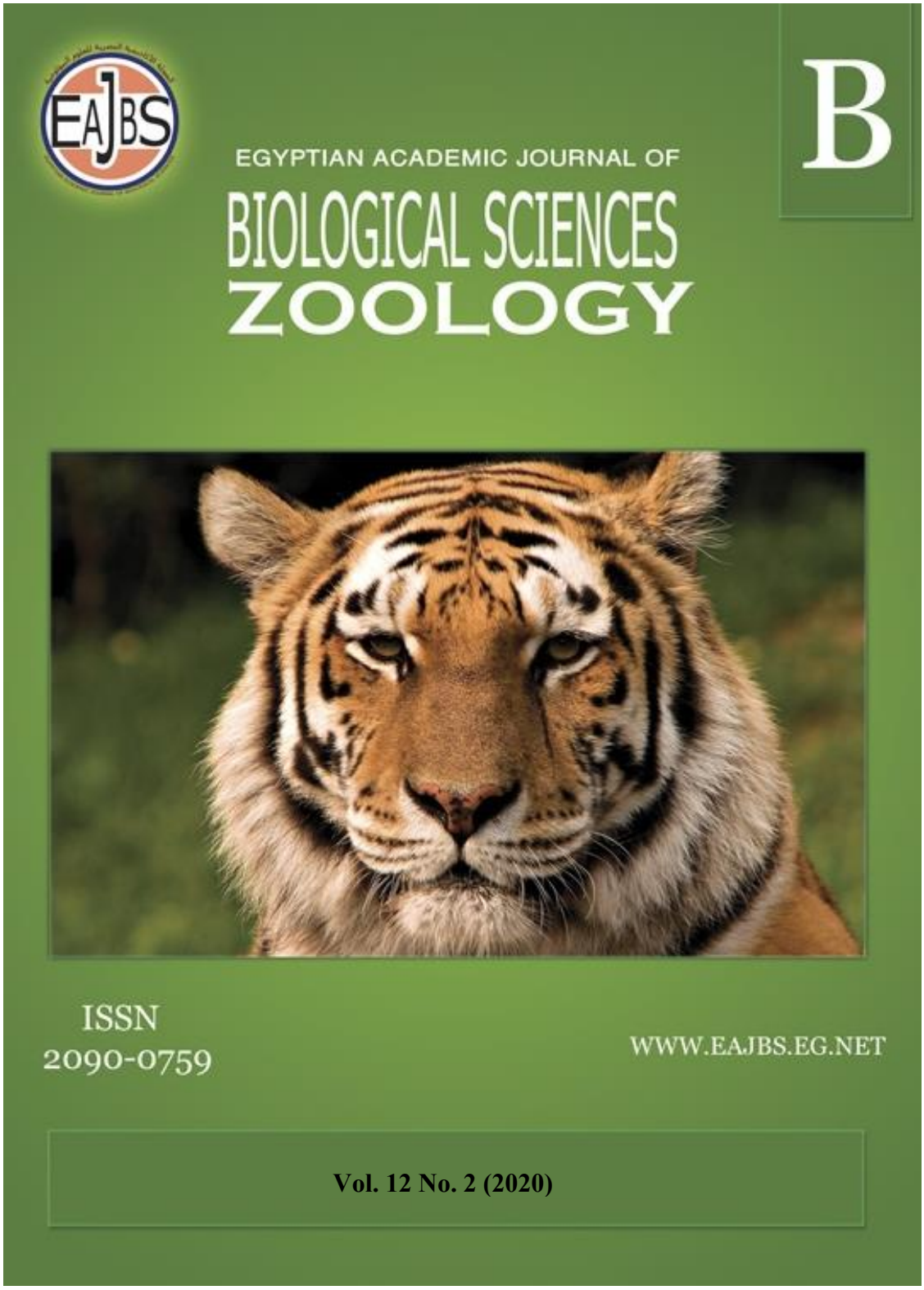

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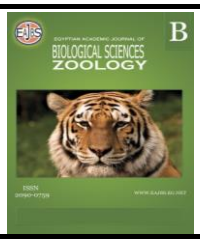

Immunological Profile of Type II Egyptian Diabetic Patients

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ABSTRACT

Background: Diabetes mellitus (DM) and its associated secondary complications are some of the leading induce of deaths worldwide. **Aim:** The aim of the study is to estimate changes in the levels of biochemical parameters as fasting blood sugar (FBS), postprandial blood sugar (PP2BS) and glycosylated hemoglobin (HbA1c); and immunological parameters as Complement proteins C3, Complement proteins C4, Immunoglobulin's (IgA, IgE, IgG, and IgM) and C-peptide of type 2 diabetic patients to obtain a full immunological and physiological profile for type 2 diabetes. **Subjects and Methods:** Blood samples from 25 type 2 diabetic patients and 5 healthy subjects were randomly selected from Banha University hospitals in Kalyubiyya, Egypt. Serum sugar and glycosylated hemoglobin were assayed by using chemical analyzer (chem 7). C3, C4, IgA, IgE, IgG, IgM and C-peptide were measured by ELISA **Results:** FBS, PP2BS, and HbA1c significant increase in type 2 diabetic patients compared to healthy subjects. C3, IgA, IgE, IgG, and C-peptide showed a significant increase in type 2 diabetic patients compared to healthy subjects. **Conclusion:** FBS, PP2BS, and HbA1c used as good tools for the diagnosis of diabetes mellitus. Type 2 diabetes in the current studied subjects is characterized by alternative pathway activation. Immunological biomarkers such as C3, IgA, IgE, and IgG were increased in type 2 diabetic patients by poor control of diabetes and long duration of disease.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2008). The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization (2003) has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million (Rowley and Bezold, 2012).

Diabetes mellitus can be classified into four types based on etiology; type 1 diabetes or insulin-dependent diabetes mellitus (IDDM), type 2 diabetes, or non-insulin dependent

diabetes mellitus (NIDDM), gestational diabetes and other types of diabetes (American Diabetes Association, 2008).

In type 2 diabetes, the cause is a combination of resistance to insulin action and defects in insulin secretion. In adults, type 2 diabetes is much more prevalent than type 1 diabetes, but it is rare in children and adolescents. Individuals with type 2 diabetes do initially not need insulin treatment to survive, and often not later in life either (American Diabetes Association, 2008).

Patients with either type 1 or type 2 diabetes are at increased risk for atherosclerotic, macrovascular disease. Macroangiopathy and cardiovascular disease account for 70-75% of deaths in people with diabetes (Grant and Davies, 2003).

Fasting glucose is directly proportional to the severity of the diabetes mellitus. During this test, blood is drawn from a vein in the patient's arm after the patient has not eaten for at least eight hours, usually in the morning before breakfast. The red blood cells are separated from the sample and the amount of glucose is measured in the remaining plasma. A plasma level of 200 mg/dl or greater strongly indicates diabetes provided that drugs such as glucocorticoids are not being administered. The fasting glucose test is usually repeated on another day to confirm the results (Ngugi *et al.,* 2012).

Post-prandial blood glucose levels are generally <120mg/dl in healthy nondiabetic subjects and rarely exceed 140mg/dl, which reflects the World Health Organization (WHO) definition (WHO, 2006).In 2010, the American Diabetes Association (ADA) recommends using HbA1c ≥6.5% for the diagnosis of diabetes and using HbA1c 5.7%-6.4% for the diagnosis of pre-diabetes. More clinicians are in favor of using HbA1c as a diagnostic tool for the following reasons (American Diabetes Association, 2010). Complement is a system of more than 30 proteins in the plasma and on cell surfaces, amounting to more than 3 g/L and constituting more than 15% of the globular fraction of plasma (Walport, 2001).

The complement system is an important component of the immune system and has a key role in facilitating the clearance of microorganisms and damaged cells by antibodies and phagocytic cells (Flyvbjerg, 2010). Activation of any one of these pathways leads to the production of complement C3 convertase, which activates complement component C3, leading to generation of the opsonic C3b and eventually generation of the membrane attack complex (MAC), which lyses, damages, or activates target cells (Turner, 2003). Serum complement component C3 is vital to the three complement activation pathways and increased inflammation. Found both C3 and C4 levels predicted inflammatory and biochemical cardiovascular risk scores in adolescents (Copenhaver *et al.,* 2020).

Immunoglobulins are produced by plasma cells, acting as a critical part of the immune response. Serum immunoglobulin concentrations were routinely measured in the clinic, since they provide key information for humoral immune (Gonzalez-Quintela, 2008).
Antibodies are secreted by B cells of the adaptive immune system, mostly by differentiated B cells called plasma cells. Antibodies can occur in two physical forms, a soluble form that is secreted from the cell to be free in the blood plasma, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B-cell receptor (BCR) (Borghesi and Milcarek, 2006).

C-peptide is often used in the clinic to monitor the beta-cell function in the diabetic patient and to differentiate between type 1 and type 2 diabetes (Marques *et al.,* 2004).
The pancreas of patients with type 1 diabetes is unable to produce insulin and therefore they will usually have a decreased level of C-peptide and insulin levels, whereas C-peptide and insulin levels in type 2 patients are normal or higher than normal (Carina *et al.,* 2001).
The aim of the study is to estimate changes in the levels of biochemical parameters as fasting blood sugar, postprandial blood sugar, and glycosylated hemoglobin; and immunological parameters as C3, C4, IgA, IgE, IgG, IgM and C-peptide of type 2 diabetic patients to obtain

a full immunological and physiological profile for type 2 diabetes in the studied patients.

MATERIALS AND METHODS

Patients:

Blood samples from 25 type 2 diabetic patients and 5 healthy subjects were randomly selected from Banha University hospitals in Kalyubiyya, Egypt.

The target subjects were divided into two groups. The target groups were Egyptian men and women who were healthy and diabetic.

Target subjects were grouped, depending on the clinical examination that was done by hospitals, for diabetes mellitus as follows:

Group 1: Twenty-five patients with type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM).

Group 2: Five healthy control subjects (the control group for type 2 diabetes).

Blood Collection:

The collections of samples were taken from April 2017 until April 2018. Consent was taken from all patients before blood sampling. 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 tetraacetic acid) for glycosylated hemoglobin (HbA1c). A third part of samples was poured into clean test tube without anticoagulant and left for 2-3 minutes in 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 blood samples were transferred in test tubes to Al-Azhar University hospitals.

Methods:

Biochemical Parameters:

Serum blood glucose was determined according to the method described by Tietz (1995) using available kits of spectrum, Egypt by Chem 7 (chemical analyzer).

Glycosylated hemoglobin (HbA1c) was determined according to the method described by Trivelli *et al.* (1971) using available kits of spectrum, Egypt by Chem 7.

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) concentration was determined by enzyme immunoassay (ELISA) according to the method described by Michel *et al.* (1980) using available kits of BioCheck, Inc, America by Beckman Coulter.

Serum C-peptide concentration was determined by enzyme immunoassay (ELISA) according to the method described by Bonger and Garcia-webb (1984) using available kits of bioactive diagnostic, Germany 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. Differences were considered statistically significant at ($P < 0.05$).

RESULTS

Blood samples from randomly selected 25 type 2 diabetic patients, 5 (20%) of these patients were males and the remaining 20 (80%) were females, age between 36 to 64 years (mean age, 50.48±1.86 years), and 5 healthy subjects without diabetes, 2 (40%) of these patients were males and the remaining 3 (60%) were females, age between 35 to 62 years (mean age, 48.2±5.08 years) from the hospital were examined (Fig. 1).

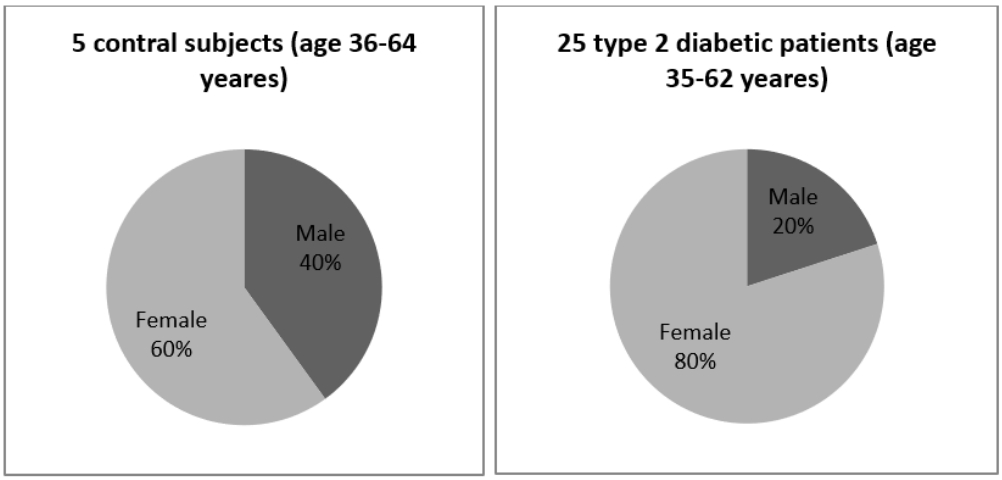

Fig. 1: Study groups for type 2 diabetes

Biochemical Parameters:

Fasting Blood Sugar (FBS): showed a highly significant increase (at $P<0.01$) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 2, where mean and S.E were (214.68±18.6) mg/dl in type 2 diabetic patients and were (79.8±3.1) mg/dl in healthy subjects. The normal range of fasting blood sugar in human plasma is 70-110mg/dl.

Table (1): The means ± SE of fasting blood sugar (FBS), postprandial blood sugar (PP2BS), glycosylated hemoglobin (HbA1c), Complement proteins C3, Complement proteins C4, IgA, IgE, IgG, IgM and C-peptide in type 2 diabetic patients and control subjects

parameters / Groups		FBS 70-110 mg/dl	PP2BS Up to140 mg/dl	HbA1c 4-6 %	C3 80-160 mg/dl	C4 20-40 mg/dl	IgA 70-400 mg/dl	IgE 0-100 IU/ml	IgG 700-1600 mg/dl	IgM 40-230 mg/dl	C-peptide 0.5-3 ng/ml
Diabetic group	Means ± SE	214.68 ± 18.6	281.5 ± 19.1	9.39 ± 0.49	192.92 ± 6.7	36.58 ± 2.28	301.62 ± 24.62	109.79 ± 16.39	1669.11 ± 65.12	202.82 ± 18.28	2.28 ± 0.11
Control group	Means ± SE	79.8 ± 3.1	98.6 ± 1.96	4.44 ± 0.05	105.8 ± 5.45	33.4 ± 1.62	181.94 ± 22.66	28.8 ± 5.09	913.56 ± 38.51	142.52 ± 18.48	1.49 ± 0.27
F-value		10.18	17.79	19.09	32.16	0.37	4.46	4.73	25.86	2.04	8.27
Probability		**	***	***	***	N.S	*	*	***	N.S	**

Significantly different at ($P<0.05$)

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

Postprandial Blood Sugar (PP2BS): showed a very highly significant increase (at P<0.001) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 2**,** where mean and S.E were (281.5±19.1) mg/dl in type 2 diabetic patients and were (98.6±1.96) mg/dl in healthy subjects. The normal range of postprandial blood sugar in human plasma is up to 140mg/dl.

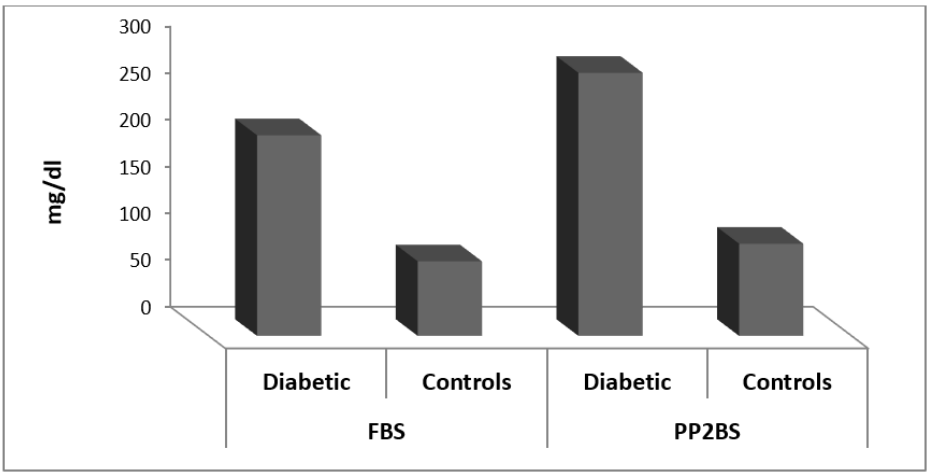

Fig. 2: The means of fasting blood sugar (FBS) and postprandial blood sugar (PP2BS) in type 2 diabetic patients and control subjects

Glycosylated Hemoglobin (HbA1c): Glycosylated hemoglobin (HbA1c) showed a very highly significant increase (at P<0.001) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 3, where mean and S.E were (9.39±0.49) % in type 2 diabetic patients and were (4.44±0.05) % in healthy subjects. The normal range of glycosylated hemoglobin (HbA1c) in humans is 4-6% (good control 4.5-7%, fair control 7.1-8.4% and uncontrolled >8.5).

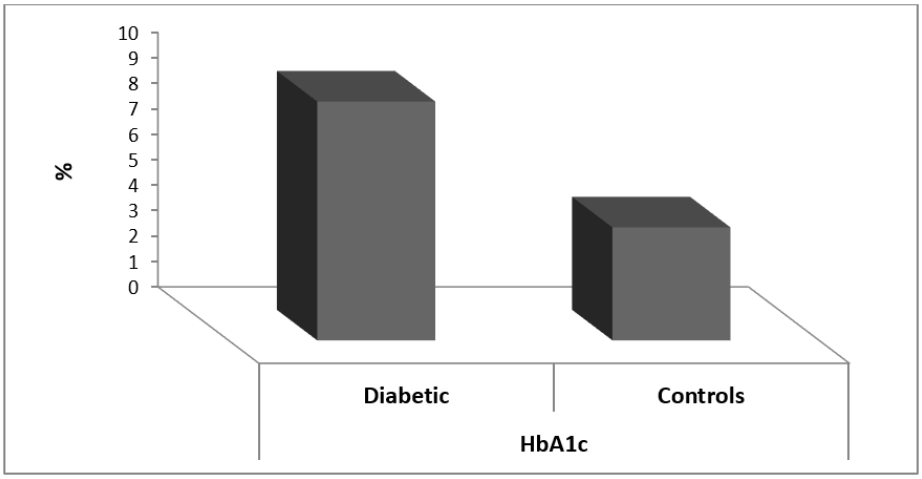

Fig 3: The means of glycosylated hemoglobin (HbA1c) in type 2 diabetic patients and control subjects

Immunological Parameters:

Levels of Serum Complement C3: showed a very highly significant increase (at P<0.001) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 4, where mean and S.E were (192.92±6.7) mg/dl in type 2 diabetic patients and were (105.8±5.45) mg/dl in healthy subjects. The normal range of C3 in human serum is 80-160 mg/dl.

Out of 25 patients, 3 cases represent (12%) had normal values of C3 while 22 cases represent (88%) had abnormal levels of C3 values as shown in Fig. 9.

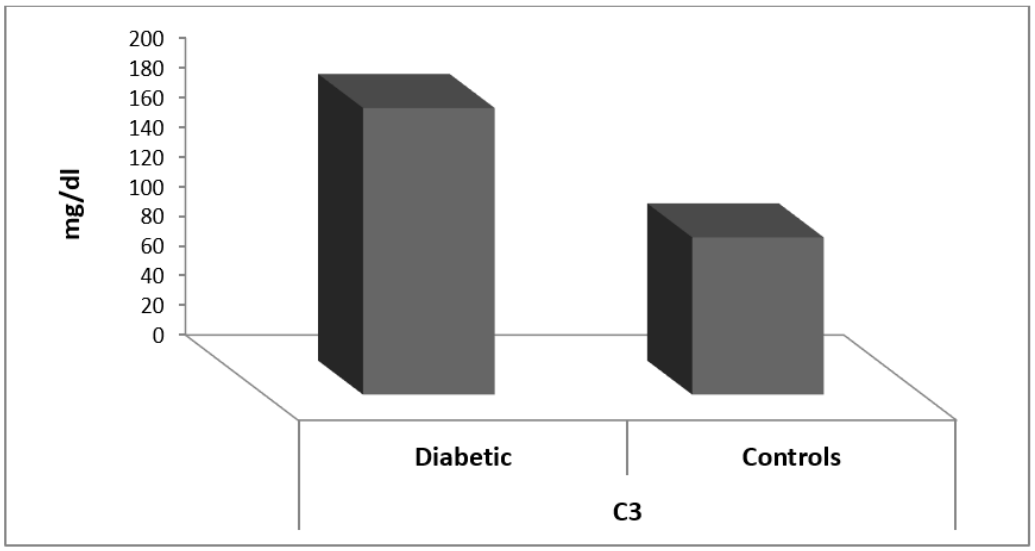

Fig 4: The means of Complement proteins C3 in type 2 diabetic patients and control subjects

Levels of Serum Complement C4: showed that, no statistically significant differences (at P<0.05) in type 2 diabetic patients compared to healthy subjects as inTable 1& Fig. 5, where mean and S.E were (36.58±2.28) mg/dl in type 2 diabetic patients and were (33.4±1.62) mg/dl in healthy subjects. The normal range of C4 in human serum is 20-40 mg/dl.
Out of 25 patients, 17 cases represent (68%) had normal values of C4 while 8 cases represent (32%) had abnormal levels of C4 values as shown in Fig. 9.

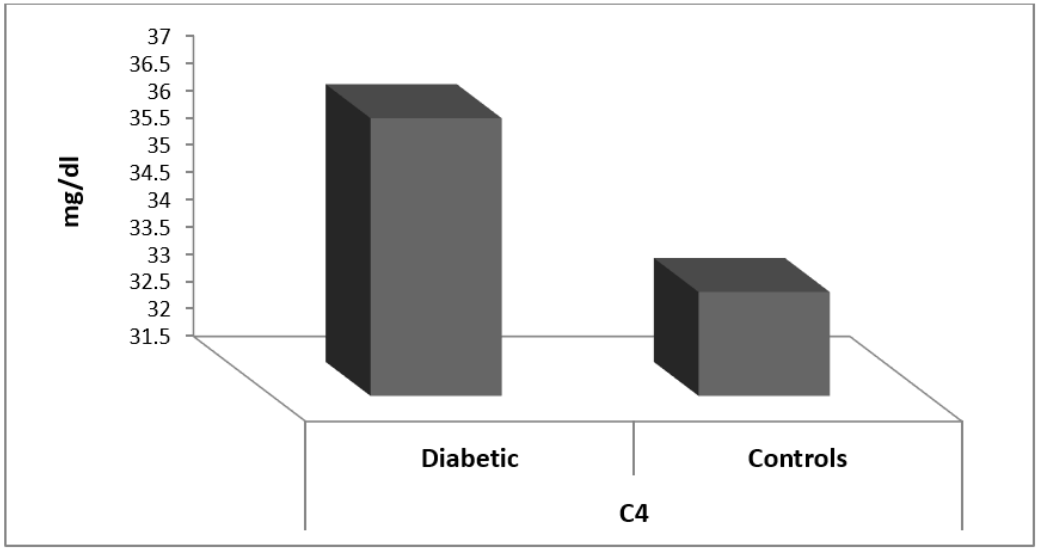

Fig. 5: The means of Complement proteins C4 in type 2 diabetic patients and control subjects

Levels of Serum Immunoglobulin A (IgA): showed a significant increase (at P<0.05) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 6, where mean and S.E were (301.62±24.62) mg/dl in type 2 diabetic patients and were (181.94±22.66) mg/dl in healthy subjects. The normal range of IgA in an adult human is 70-400 mg/dl.

Out of 25 patients, 19 cases represent (76%) had normal values of IgA while 6 cases represent (24%) had abnormal levels of IgA values as shown in Fig. 9.

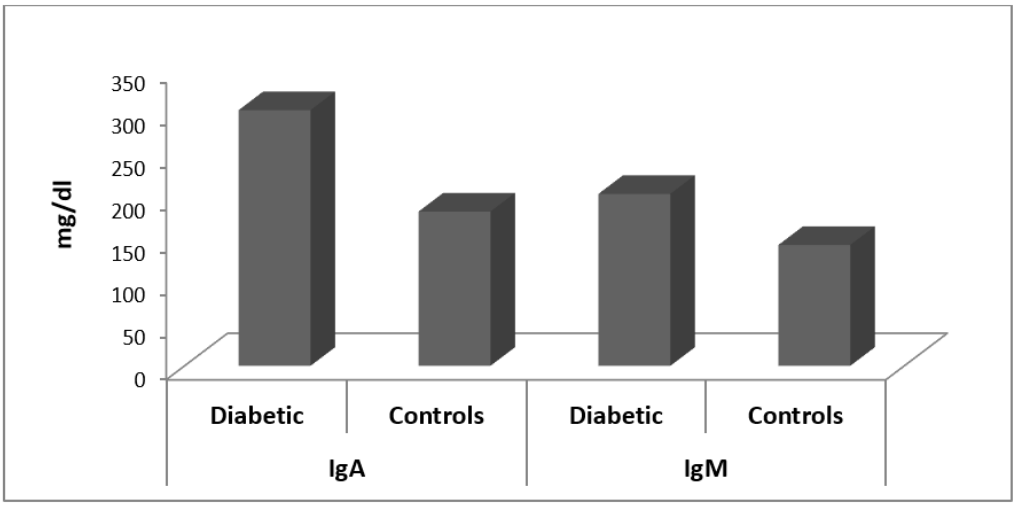

Fig. 6: The means of serum immunoglobulin A (IgA) and immunoglobulin M (IgM) in type 2 diabetic patients and control subjects

Levels of Serum Immunoglobulin E (IgE): showed a significant increase (at $P<0.05$) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 7, where mean and S.E were (109.79±16.39) IU/ml in type 2 diabetic patients and were (28.8±5.09) IU/ml in healthy subjects. The normal range of IgE in human serum is 0-100 IU/ml.

Out of 25 patients, 11 cases represent (44%) had normal values of IgE while 14 cases represent (56%) had abnormal levels of IgE values as shown in Fig. 9.

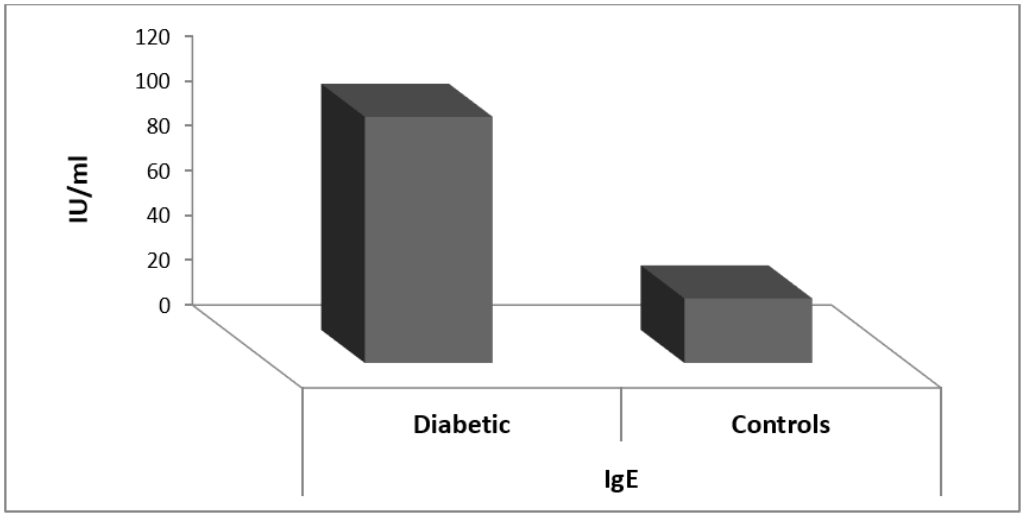

Fig. 7: The means of serum immunoglobulin E (IgE) in type 2 diabetic patients and control subjects

Levels of Serum Immunoglobulin G (IgG): showed a very highly significant increase (at $P<0.001$) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 8, where mean and S.E were (1669.11±65.12) mg/dl in type 2 diabetic patients and were (913.56±38.51) mg/dl in healthy subjects. The normal range of IgG in an adult human is 700-1600 mg/dl.

Out of 25 patients, 8 cases represent (32%) had normal values of IgG while 17 cases represent (68%) had abnormal levels of IgG values as shown in Fig. 9.

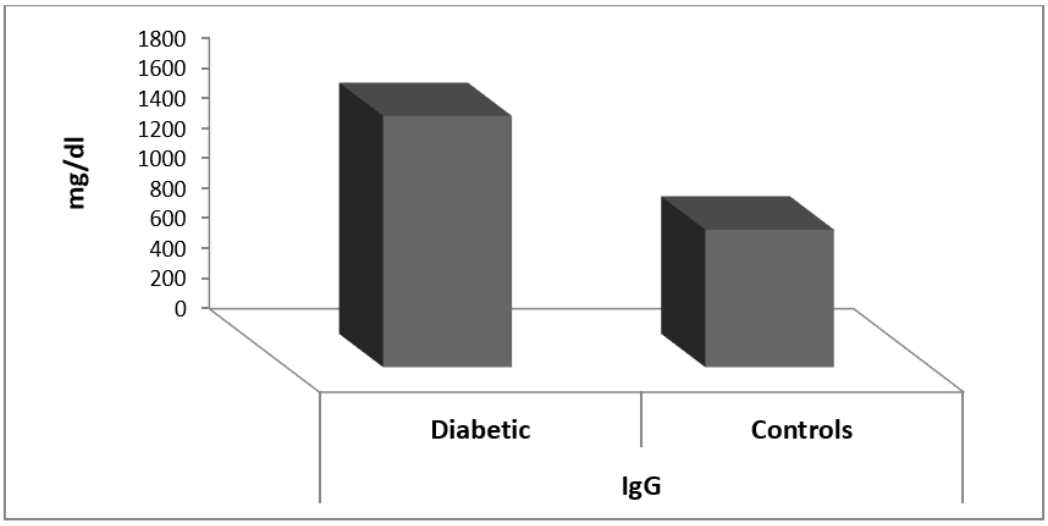

Fig. 8: The means of serum immunoglobulin G (IgG) in type 2 diabetic patients and control subjects

Levels of Serum Immunoglobulin M (IgM): showed that, no statistically significant differences (at P<0.05) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 6, where mean and S.E were (202.82±18.28) mg/dl in type 2 diabetic patients and were (142.52±18.48) mg/dl in healthy subjects. The normal range of IgM in an adult human is 40-230 mg/dl.

Out of 25 patients, 19 cases represent (76%) had normal values of IgM while 6 cases represent (24%) had abnormal levels of IgM values as shown in Fig. 9.

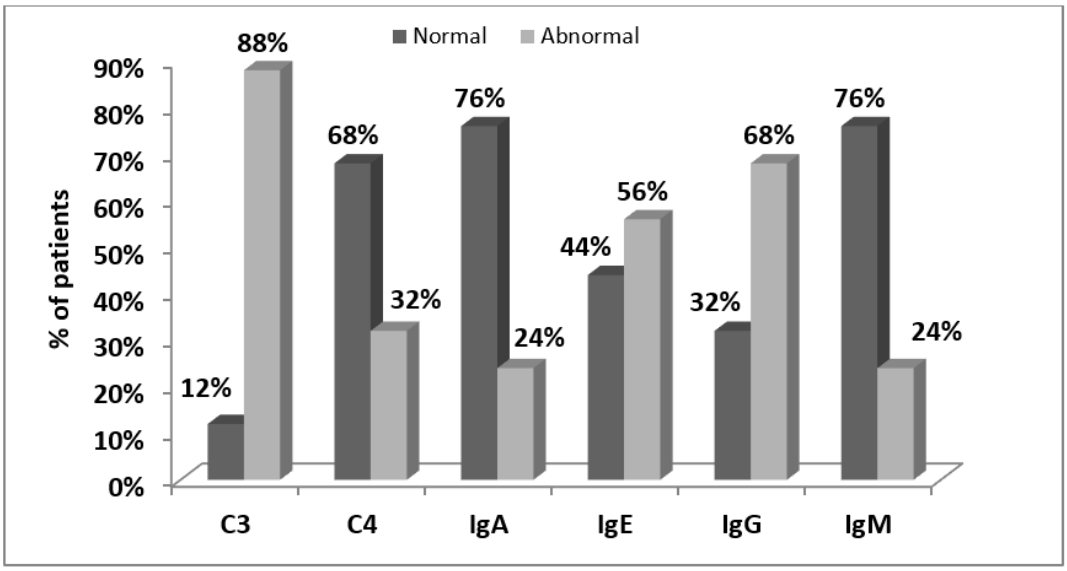

Fig. 9: Normal and abnormal levels of C3, C4, IgA, IgE, IgG, and IgM in type 2 diabetic patients

Levels of Serum C-peptide: showed a highly significant increase (at P<0.01) in type 2 diabetic patients compared to healthy subjects as in Table 1 & Fig. 10, where mean and S.E were (2.28±0.11) ng/ml in type 2 diabetic patients and were (1.49±0.27) ng/ml in healthy subjects. The normal range of serum C-peptide in human serum is 0.5-3 ng/ml.

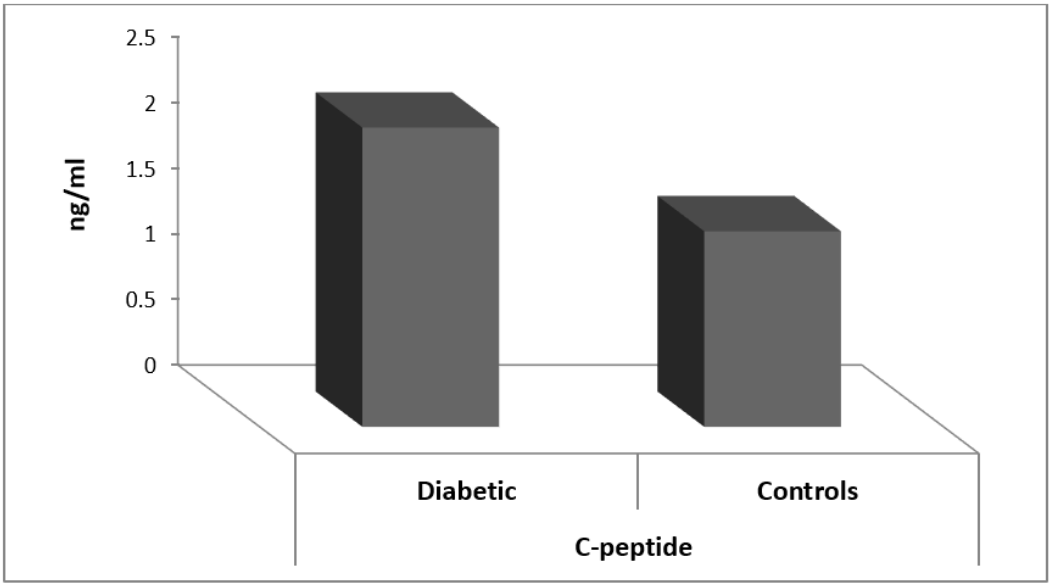

Fig. 10: The means of serum C-peptide in type 2 diabetic patients and control subjects

DISCUSSION

Fasting Blood Sugar, Postprandial Blood Sugar and Glycosylated Hemoglobin (HbA1c):

The present study 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 2 diabetic patients compared to healthy subjects. This may be due to insufficient production of insulin by ß-cells of islets of Langerhans in the pancreas.

This result was in agreement with those recorded by several authors. Narayanan *et al.* (2020) observed that HbA1c value correlated significantly with FBS level in type 2 diabetes mellitus patients. The factor significantly affecting the HbA1c value is the fasting blood sugar level. Alzahrani *et al.* (2019) recorded that the females had significantly higher values for HbA1c, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) compared to the males. Kharroubi *et al.* (2014) found that mean HbA1c and FPG increase significantly with increasing body mass index in diabetic patients. Tanaka (2012) showed that elevated plasma glucose levels, both fasting and 2-h postprandial, can be considered as risk factors for vascular complications in patients with type 2 diabetes mellitus. Shrestha *et al.* (2012) suggested that postprandial blood glucose correlated better than fasting blood glucose to HbA1c. Both postprandial blood glucose and fasting blood glucose significantly correlated with HbA1c. Pasupathi *et al.* (2010) recorded that the level of blood glucose, HbA1c and mean blood glucose (MBG) was significantly increased in diabetics than non-diabetic subjects. There was a statistically significant relationship between MBG and HbA1c. Norbergim *et al.* (2006) reported that FPG $\geq$ 6.1mmol L^{-1} and HbA1c $\geq$ 4.7%, had sensitivities and specificities. The combination of HbA1c, FPG, and BMI is effective in screening for individuals at risk of future clinical diagnosis of type 2 diabetes. Perry *et al.* (2001) reported that the subjects with OGTT-diagnosed diabetes and FPG levels between 5.5 and 8.0mmol/l, detection of an elevated HbA1c. HbA1c measurement improves the sensitivity of screening in high-risk individuals.

The obtained result was in disagreement with Ju *et al.* (2014) who showed that fasting blood sugar, 2-hour postprandial blood sugar, and glycosylated hemoglobin levels were not significantly different in patients with type 2 diabetes from three different altitudes.

Moreover, Yu *et al.* (2012) reported that significantly higher HbA1c levels and lower levels of fasting and 2-hour PG than the latter group.

Complement Proteins C3 and C4:

The present work revealed that complement proteins C3 showed a very highly significant increase (at $P<0.001$) and complement proteins C4 showed no statistically significant differences (at $P<0.05$) in type 2 diabetic patients compared to healthy subjects. The increase in the levels of complement proteins C3 indicated activation of the complement system in type 2 diabetic patients. The increased levels of the only C3 when C4 is normal to indicate alternative pathway activation of the complement system.

The results were in accordance with the findings of Jian-bin *et al.* (2020) who recorded that serum complement C3 was also positively correlated with C-peptide during the oral glucose tolerance test (OGTT) at baseline. Serum complement C3 was independently associated with the index of islet β-cell function at baseline in patients with T2D. Zhang *et al.* (2019) showed that at different levels of proteinuria, increased C3 levels were independent indicators of non-diabetic renal disease (NDRD) in T2DM. Mustafa *et al.* (2019) found that a significant increase in serum C3 levels of patients compared with the control group but C4 level did not show any significant change for all groups. The inflammation process associated with T2DM where inflammatory markers (C3 and CRP) elevated. Peake *et al.* (2005) suggested that elevated levels of C3 and factor B in the diabetic relative's group may have resulted from increased synthesis by adipose tissue. C4 did not differ between the diabetic group and the control group. Also, Engstrom *et al.* (2005) found that elevated levels of C3 were associated with an increased incidence of diabetes in middle-aged men.

These findings results were nearly similar to those of Al-Sowayan (2015) and Bergamaschini *et al.* (1991) who recorded that C3 and C4 levels were not significantly different in IDDM and NIDDM patients than in age-matched controls. Moreover, Morimoto *et al.* (1988a) and McMillan (1980) recorded that C3 and C4 were significantly elevated in subjects with non-insulin-dependent diabetes mellitus (NIDDM) as compared with healthy non-diabetic controls. The elevation of all complement components was found to be unrelated to the presence or severity of diabetic microvascular sequelae. Also, Morimoto *et al.* (1988b) found that CH50, C3, and C4 significantly increased in both IDDM and NIDDM compared with non-diabetic healthy controls. These observations suggested that there is a high level of complements in both types of diabetes mellitus, but the complement activation seems to be much enhanced in IDDM compared with NIDDM.

The obtained result was in disagreement with Saleh (2011) who recorded that the altered levels of serum complement and immunoglobulins might be responsible for depressed immune response in patients with type 2 diabetes. Besides, Islam *et al.* (2006) suggested that complement component C4 was found to be significantly elevated whereas the levels of C3 were slightly lowered.

Immunoglobulin's (Igs):

The present work revealed that immunoglobulin A (IgA) showed a significant increase (at $P<0.05$), immunoglobulin E (IgE) showed a significant increase (at $P<0.05$), immunoglobulin G (IgG) showed a very highly significant increase (at $P<0.001$) and immunoglobulin M (IgM) showed that no statistically significant differences (at $P<0.05$) in type 2 diabetic patients compared to healthy subjects. This may be due to poor control of diabetes and long duration of disease.

These results also were in agreement with Wang *et al.* (2017) who showed that elevated IgA and IgE levels were positively associated with the prevalence of prediabetes, and IgM had a trending association with prediabetes prevalence in the adult population. The findings suggested that immunoglobulins might contribute to prediabetes. Botchey (2014) showed that serum IgA and IgG were significantly higher in type 2 diabetics than in the non-

diabetics but there was no significant difference in IgM between the two groups. Weng *et al.* (2012) found that no significant differences between the diabetic nephropathy (DN) and non-diabetic renal disease (NDRD) groups with respect to serum levels of IgM. However, there were significant differences between the two groups with respect to serum IgG and IgE. Mistry and Kalia (2008) recorded that serum IgG and urinary IgG excretion were increased significantly in diabetic patients compared to healthy controls, which were further increased significantly in chronic renal failure patients with respect to the clinical stage of nephropathy. Rodriguez-Segade *et al.* (1996) recorded that an increase in circulating IgA concentrations is a generalized phenomenon among diabetic patients; IgA concentrations above the reference range are more common among male than female diabetics, and diabetic complications are associated with a significant increase in serum IgA concentration.

These finding results were nearly similar with those of Ardawi *et al.* (1994) and Rodriguez-Segade *et al.* (1991) who concluded that abnormal levels of IgA, IgG, and IgM are very common in diabetic patients in whom serum IgA concentrations are influenced by the degree of glycaemic control. Whether changes in IgA and other immunoglobulins are implicated in the pathogenesis of diabetic complications.

C-peptide:

The represented data showed that C-peptide showed a highly significant increase (at $P<0.01$) in type 2 diabetic patients compared to healthy subjects may be due to the weakening of ß-cells of islets of Langerhans in the pancreas.

This result was in agreement with Cho *et al.* (2014) who reported that serum C-peptide level was significantly lower in children with T1DM than in children with T2DM. Serum C-peptide level measured at initial diabetes diagnosis is significantly useful for classifying DM type and choosing the appropriate treatment method. Bo *et al.* (2012) found that higher fasting concentrations of C-peptide are associated with a reduced risk for several chronic complications but not all-cause or specific-cause mortality in type 2 diabetic patients. Abdullah *et al.* (2010) reported that the fasting C-peptide levels are useful in type 2 diabetic patients with poor glycemic control to assess the endogenous insulin reserve and to alter the modality of treatment. Obese patients had higher C-peptide levels compared to non-obese patients. Siraj *et al.* (2002) recorded that C-peptide levels are good discriminators between type 1 and type 2 diabetes and may also be useful in identifying subjects with type 2 diabetes who require insulin therapy. The mean basal C-peptide level was higher in type 2 diabetic subjects than control subjects.

Conclusion

The present study comes to a conclusion that: FBS, PP2BS, and HbA1c used as good tools for the diagnosis of diabetes mellitus. Complement proteins C3 showed a significant increase and complement proteins C4 showed no statistically significant differences in type 2 diabetic patients compared to healthy subjects. Type 2 diabetes in the current studied subjects is characterized by alternative pathway activation. IgA, IgE, IgG, and C-peptide showed a significant increase in type 2 diabetic patients compared to healthy subjects. Immunological biomarkers such as C3, IgA, IgE, and IgG were increased in type 2 diabetic patients by poor control of diabetes and long duration of disease. Immunological biomarkers profile might help in prevention and control of diabetes.

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