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Evaluation of HbA1c, C-peptide and Rf in DM patients related to COVID-19, ABO group and BMI in Babylon, Iraq

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Abstract

Background: A total of 30(100%) cases of diabetes mellitus DM were collected from a private laboratory during the period from October 2023 to February 2024, compared with 30 control to evaluate several parameters in a case-control study.

The demographic information of sex distribution for DM cases appear 16(53%) male and 14(47%) and the mean±SD of the age was 45.6±14.7 compared to the control 33.6±10.5 which reveals a significant at P = 0.0006. The mean±SD of the BMI was 29±6.4 in DM cases compared to the control 25.8±3.4 which reveals a significant difference at P = 0.0187.

Distribution of DM types appears in 16(53%), and 14(47%) cases of DM type 1 and 2 respectively with no significant difference between the two study groups. The mean±SD of HbA1c Level with DM Patients and was 9.2±2.8 compared to the control 5.6±0.9 which appeared a significant at P = 0.0002.

Methods: Evaluation of C-peptide Level appears the mean±SD of the C-peptide for DM cases was 5.7±4.5 compared to the control 3.3±2.4. this case-control study appeared a significant difference at P = 0.0125. RBS Level mean±SD of DM cases was 275.7±121.5 compared to the control 103.7±16.6. this case-control study appeared significant at P = 0.0001. The distribution of DM cases in blood groups O+, A+, B+, AB+, AB-, and O- was 10(33.3%), 8(26.7%), 5(16.7%), 3(10%), 3(10%), 1(3.3%), respectively.

Results: All the cases in the present study show negative results for rheumatoid factor and there is no correlation between RF and DM. 2(7%) DM cases have a cardiac problem while the other 28(93%) don't suffer from any cardiac problem. Relationship between COVID-19 and DM Type-1 Patients appears 15(50%) have positive COVID-19, 12(40%) has DM type 1 and 3(10%) has DM type-2.

Conclusion: The study was concluded an appositive relationship between age, HbA1c, C-peptide, RBS, O+ and A+ blood groups and developments of diabetes mellitus.

Keywords: Diabetes; C-peptide; Blood groups; Covid-19

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1. Introduction

Hyperglycemia caused by insulin resistance; insufficient insulin secretion; or excessive glucagon secretion is a hallmark of diabetes mellitus; a collection of physiological dysfunctions. Destruction of pancreatic beta cells occurs in type 1 diabetes (T1D); an autoimmune condition. The increasingly worse glucose management caused by insulin resistance and malfunctioning pancreatic beta cells is the main issue with the far more prevalent type 2 diabetes (T2D) (1).

In a clinical investigation including Chinese adults; researchers found that a high body mass index (BMI) was linked to an increased risk of developing type 2 diabetes. In the Asian old population with type 2 diabetes mellitus; a higher body mass index was linked to worse insulin sensitivity and more insulin resistance (2).

One of the most useful markers of long-term glycemic control is hemoglobin A1c; which can show the whole glycemic history of the last 2-3 months. The hemoglobin A1c (HbA1c) level is a good predictor of the likelihood of diabetes-related problems in the future and a valid indicator of chronic hyperglycemia. Because of the wealth of information; it provides; HbA1c has become a trusted biomarker for diabetes diagnosis and prognosis (3).

Separate associations with COVID-19 severity and greater mortality were shown to be present in individuals with diabetes mellitus and varying degrees of hyperglycemia (4); are higher in patients with diabetes mellitus; according to multiple population-based research. Additionally; COVID-19 mortality is increased when usual diabetes mellitus comorbidities such as cardiovascular disease; heart failure; and chronic renal disease are present: (5). As a marker of pancreatic beta cell activity; C-peptide has found widespread application. It has the same molecular formula as endogenous insulin but has a longer half-life and remains in the body at a constant concentration (6).

In Iraq; there are insufficient epidemiological studies and randomized controlled trials (RCTs) related to diabetes; therefore; it remains difficult to fully understand the prevalence of diabetes in Iraq and the most effective therapies for the Iraqi population (7).

Aim of the study

- Detection of the relationship between demographic data (age; sex; BMI) and Diabetes mellitus type1; 2 .
- Evaluation of the reality of C-peptide in the diagnosis of DM-1.
- Investigation of the role of HPA1c as a potential diagnosis of DM - 1; 2.
- Detection the relationship between DM-1; 2 and post COVID-19 infection.

2. Material and methods

2.1. Study design

The present study was designed as a case-control study.

2.2. Patients or subjects

From October 2022 through February 2023, blood samples were obtained from private laboratories in Babylon City. The study included 30 samples from random DM patients, and 30 samples from healthy people were collected in EDTA-coated tubes and were stored at 4 C° until analysis.

Rheumatoid latex factor kit is used to determine rheumatoid factor levels in different samples manufactured by Spinreact / Spain Company.

2.3. Principle of the method

It is possible to detect RF in human serum in a qualitative and semi-quantitative manner using the RF-latex, a slide agglutination test. Addition of RF to latex particles coated with human gammaglobulin causes agglutination.

2.4. Procedure

- The samples and reagents were left to cool to room temperature.
- Separate circles on the slide test were used to deposit fifty microliters of the sample as well as one drop of each control (positive and negative).
- Prior to use, the RF-latex reagent was thoroughly mixed, and 50 µL of a single drop was added.

- Each sample was mixed with a separate stirrer after the drips were spread out across the entire circle.
- For two minutes, the slide was turned very slowly.
- Value for reference: normal saline is required for mitigation when the result is up to 8 IU/ml, whereas a negative result demands less than 8 IU/ml.

2.5. HBA1C kit

2.5.1. Principle of the method

This HbA1c Test Kit measures the concentration of hemoglobin A1c in blood using fluorescence immunoassay technology. Detector HbA1c antibodies that are fluorescently labeled bind to HbA1c in blood samples. The HbA1c antibody mounted on the test strip catches the complexes formed between the detector antibody and HbA1c. A direct correlation between HbA1c concentrations in blood samples and the fluorescence signal intensity has been established. One way to measure HbA1c is by comparing the ratio of its fluorescent signals.

2.5.2. Procedure

The instructions of the procedure were followed, as it was recommended by the manufacturer.

- Add 5 ul sample to diluent buffer
- After mixing the sample thoroughly, incubated the sample at room temperature for 1 min.
- After 1 min lysis, transferred 70 ul of the mixture to the test card.
- Incubated the test card at 25c° for 12 min.
- After incubation, the machine automatically runs the test.

2.6. C-peptide kit

2.6.1. Principle of the method

Detects typical human illnesses by means of immunochromatographic technique. Automatic calculations are made from the triggered fluorescence at the test line, which is based on specific antigen-antibody reactions.

2.6.2. Procedure

The instructions of the procedure were followed, as it was recommended by the manufacturer.

- The test card should be left at room temperature (20-25°C) after being removed from the refrigerator.
- Follow the on-screen prompts to power up the device. With the timer set to 15 minutes and the temperature at 25°C, open the incubator.
- Verify that the diagnostic kits' lot numbers and ID chips match up. During the insertion process, avoid touching the end of the ID chip.
- Open the foil pouch by tearing along the splice and place the test card on the flat operation table. The test card should be used within 1 hour.
- Add 70-µL sample on the test card.
- Place test card into the incubator for 15 minutes before removing
- Place the test card into the analyzer's designated slot and begin testing in accordance with the instructions provided. It will automatically display the outcome on the screen a few seconds later. The outcome can also be printed out and saved.

2.6.3. Random Blood Sugar

Uses to determine Blood Sugar levels in different samples, manufactured by spinreact / spain company.

- Allow the samples and reagents to cool to room temperature.
- Transfer the contents of the pipette tips into the designated tubes:

CAL. Standard	Sample	Blank	TUBES
1.0 mL	1.0 mL	1.0 mL	R1.Monoreagent Sample CAL. Standard
-	1.0 mL	-	
10 µL	-	-	

- Combine, then set the tubes aside for either 5 minutes at 37 degrees Celsius or 10 minutes at room temperature.
- Compare the samples' and standard's absorbance (A) at 500 nm to that of the reagent blank.

With protection from light, the color will last for around two hours.

2.7. Calculations

$$\frac{A\ sample}{A\ standard} \text{ x C Standard = mg/dL glucose}$$

For reanalysis, dilute samples with saline to a ratio of 1:4 if their concentrations are greater than 500 mg/dL. Add together all the outcomes and divide by Young (2000). If you want to represent the results in SI units, you can do it like this: mg/dL x 0.0555 = mmol/L.

Body mass index calculation:

Body mass index was calculated by equilibrium:

$BMI=weight\ length\mathbf{2}$ (Nuttall, 2015).

2.8. Statistical Analysis

The linear regression test (linear regression) expresses the relationships between enzyme linear activities and semen parameters through linear equations and correlation coefficients for each relationship. The known statistical system, statistical package for the social sciences (SPSS) (version 16.0), was used to infer the significance, mean, and standard error (SE) of these relationships, these parameters represent the size of the two axes and the type of linear relations

3. Results and discussion

3.1. Distribution of Sex in DM Patients

Figure 1 revealed out of 30(100%) diabetes mellitus patients, there were 16(53%) male and 14(47%) female.

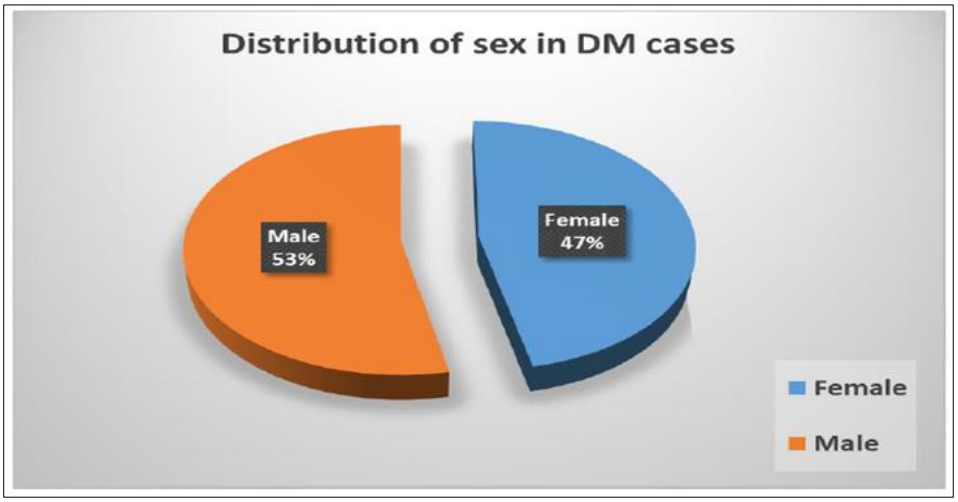

Figure 1 Distribution of sex in DM cases

The current finding showed that there were no statistically significant variations between the sexes in terms of the prevalence of DM, while the percentage of cases was higher in men. The results showing that the male present with T1DM was higher than that of females While other study (8) demonstrated no effect of sex on patients with DM. Also new clinical research showed that Gender and duration of diabetes didn't show any significant differences in effect on patients (9).

Although there is some indication that adult men may have a higher risk of developing diabetes than women do, the available data points to distinct patterns of fat storage in men and women as a possible explanation (10)

3.2. Distribution of Age in DM Patients

Out of 30(100%) diabetes mellitus patients, the mean±SD of the age of DM cases was 45.6±14.7 compared to the control 33.6±10.5. this case-control study appeared a significant difference between the two study groups where P = 0.0006 that explain in table 1.

Table 1 Distribution of Age in DM Patients and control group

Study groups	Mean ± SD	P value
DM case n=30	45.6 ± 14.7	0.0006
Control n=30	33.6 ± 10.5	

The majority of Chinese adults with diabetes or prediabetes are above the age of 40, according to the current study's findings. In the 40-49 age group, 11.1% of people have diabetes and 40.3% have prediabetes; in the 60-69 age group, however, these rates have risen to 47.6% and 23.9%, respectively (11).

Clinical study shows among the diabetes mellitus patients the age ranged from 21 years to 80 years, the lowest diabetes mellitus incidence was among (the 71-80) years old age group (1%) followed by (the 20-30) year's old age group (6%), whereas the highest diabetes mellitus incidence was among (41-50) years old age group (34%) (11). It was substantially greater in males (p value 0.0005) and the average age at first diagnosis was 15.3±9 years in an Iraqi study (12).

3.3. Distribution of BMI in DM Patients

Table 2 showed out of 30(100%) diabetes mellitus patients, the mean±SD of the BMI of DM cases was 29±6.4 compared to the control 25.8±3.4. this case-control study appeared a significant difference between the two study groups where P = 0.0187.

Table 2 Distribution of BMI in DM Patients and control group

Study groups	Mean ± SD	P value
DM case n=30	29 ± 6.4	0.0187
Control n=30	25.8 ± 4.4	

Longitudinal data that was representative of the nation was used for the analysis. The physician's report is crucial for the diagnosis of diabetes, its consequences, and insulin dependency. In order to ascertain if an individual had a history of DM diagnosis, we utilized an average review time of about 4 years. On average, people with diabetes and its complications were followed for around seven years after the diagnosis (13).

3.4. Distribution of DM types

Fifteen (53% of the total) diabetic patients had type 1 diabetes and fourteen (47% of the total) had type 2 diabetes. Neither of the research groups differed significantly from the other which revealed in figure 2.

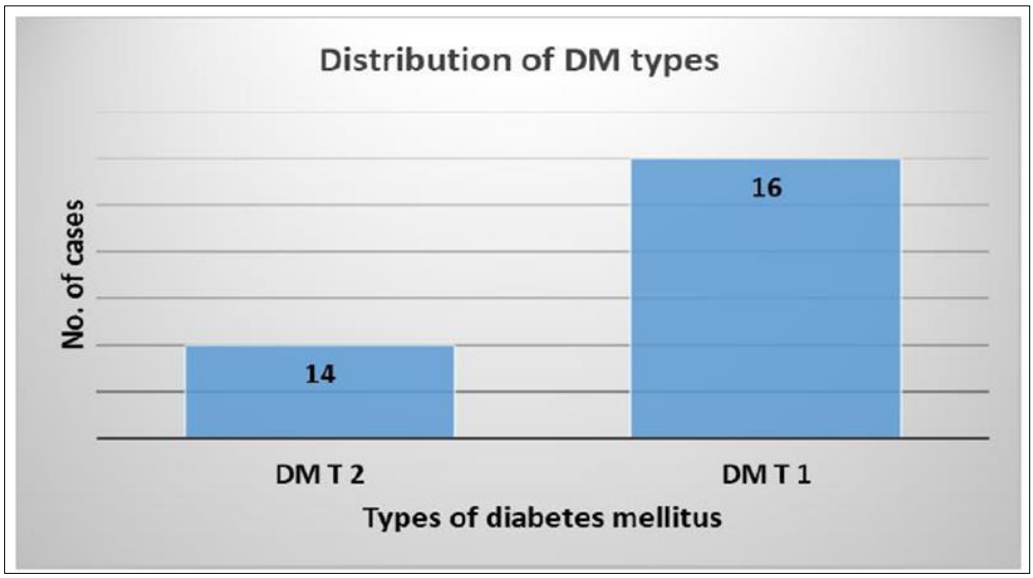

Figure 2 Distribution of DM types in study cases

Type 2 diabetes is more common than type 1 in Iraq, according to recent studies. The disease is more common in women and those older than 44 years old. Type 2 diabetes was more common in adults aged 44 and above, whereas type 1 was more common in those aged 15–44, according to the study (14).

3.5. Evaluation of HbA1c Level with DM Patients and Control

The result explains in table 3, out of 30(100%) diabetes mellitus patients, the mean±SD of the HbA1c for DM cases was 9.2±2.8 compared to the control 5.6±0.9. this case-control study appeared a significant difference between the two study groups where P = 0.0002.

Table 3 Distribution of HbA1c in DM Patients and control group

Study groups	Mean ± SD	P value
DM case n=30	9.2 ± 2.8	0.0002
Control n=30	5.6 ± 0.9	

The present result shows HbA1c values are crucial not only for diabetes monitoring. HbA1c has been demonstrated a significant rise in levels in diabetic patient groups than control groups, for both genders and age groups. According to new research, hyperglycemia test results with a HbA1c level greater than 7% are abnormal in over 90% of diabetic individuals (15).

Other clinical research reveals that an increase in the mean levels of HbA1c (7.84%) and a significant rise in HbA1c levels, compare between patient and the control groups at Al-Yarmouk Teaching Hospital in Baghdad, Iraq (16).

Hyperglycemia test results of HbA1c > 7% were observed in over 90% of diabetic patients (17). Raised HbA1c levels are a leading indicator of inadequate glycemic management, which in turn exacerbates diabetic problems over time (18).

3.6. Evaluation of C-peptide Level with DM Patients and Control

Table 4 explains 30(100%) diabetes mellitus patients, the mean±SD of the C-peptide for DM cases was 5.7±4.5 compared to the control 3.3±2.4. this case-control study appeared a significant difference between the two study groups where P = 0.0125.

Table 4 Distribution of C-peptide in DM Patients and control group

Study groups	Mean ± SD	P value
DM case n=30	5.7 ± 4.5	0.0125
Control n=30	3.3 ± 2.4	

The current study found that C-peptide levels in type 2 diabetic patients were normal or slightly higher than normal, but in type 1 diabetic patients they are often lower, this is due to the fact that insulin production is severely impaired or completely absent in the pancreas of individuals diagnosed with type 1 diabetes, which means that the pancreas is unable to produce insulin. Consequently, there is typically a decrease in both C-peptide and insulin levels, as these two components are secreted in equal amounts. Even though insulin-resistant type 2 diabetics' pancreatic beta cells work normally and produce insulin, their levels of C-peptide and insulin are typically normal—or even higher than normal—due to the pancreas producing an excess of insulin due to the patients' resistance to insulin (19).

3.7. Evaluation of RBS Level with DM Patients and Control

The results showed in table 5 30(100%) diabetes mellitus patients, the mean±SD of the RBS for DM cases was 275.7±121.5 compared to the control 103.7±16.6. this case-control study appeared a significant difference between the two study groups where the P = 0.0001.

Table 5 Distribution of RBS in DM Patients and control group

Study groups	Mean ± SD	P value
DM case n=30	275.7 ± 121.5	0.0001
Control n=30	103.7 ± 16.6	

According to this research, hypercalcemia and diabetes mellitus go hand in hand. either because insulin production is inadequate or because insulin is not recognized by the cells. Hyperglycemia has a significant role in the development of such problems.

The hallmark of type 2 diabetes is recurrent hyperglycemia, patients are diagnosed with diabetes if they exhibit any one of the following symptoms: Diabetic symptoms along with random blood sugar levels of ≥200 mg/dL (11.1 mmol/L), fasting blood sugar levels of ≥126 mg/dL (7 mmol/L), or 2-hour plasma glucose levels of ≥200 mg/dL (11.1 mmol/L) during a glucose tolerance test (GTT) (20).

Patients with type I and type II diabetes had considerably higher levels of fasting blood sugar, red blood cell saturation, hemoglobin A1c, triglycerides, and total cholesterol when compared to healthy controls, according to prior research. Both type I and type II diabetic patients had noticeably decreased levels of HDL and serum thiamine compared to the control group (21).

3.8. Distribution of ABO group in DM cases

Figure 3 showed out of 30(100%) diabetes mellitus patients, the distribution of DM cases in blood groups O+, A+, B+, AB+, AB-, and O- was 10(33.3%), 8(26.7%), 5(16.7%), 3(10%), 3(10%), 1(3.3%), respectively.

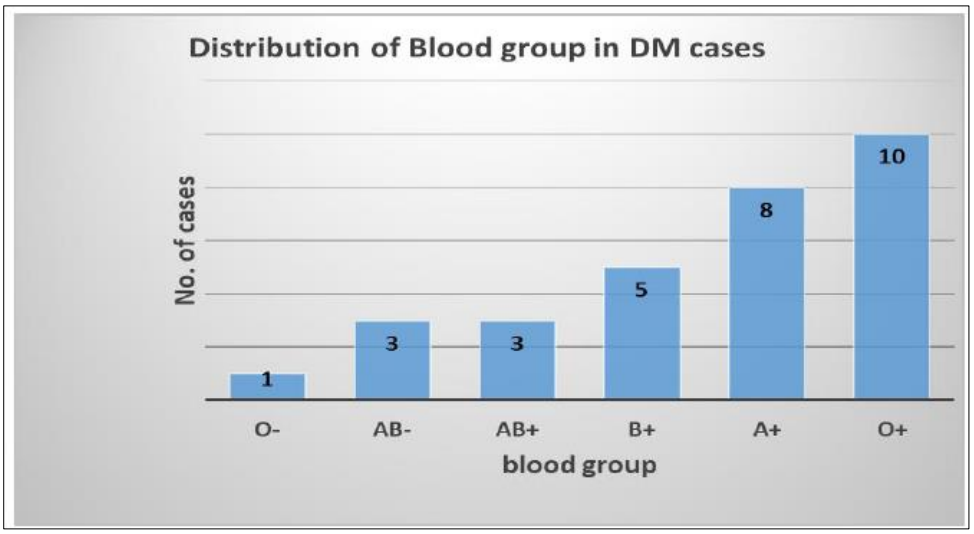

Figure 3 Distribution of blood groups in DM cases

The current research reveals a higher number of instances in the O and A blood types. Research has linked type 2 diabetes to the A and O blood types. An additional investigation revealed, however, that A and O blood types were inversely linked to type 2 DM ($p < 0.05$) in a greater proportion than those in groups A and O who did not have diabetes. Type 2 diabetes and blood types B and AB were not significantly associated ($P = 0.423$ and $P = 0.095$, respectively).

Statistical significance was not reached, but it was found that the proportion of blood group B was higher among DM 2 patients (53.71%) compared to the control group (22.52%). There is no correlation between type DM and the ABO or O blood types, according to the results. Hybrid communities, generated by recent population mixing, make up the majority of the populations where evidence of a link between genetic markers and type 2 diabetes has been established (22).

Studies examining the correlation between disease and ABO blood type distribution have shown mixed results, with some finding no correlation and others finding a positive one. Even though it's less prevalent among diabetics. Our findings are intriguing because they suggest a protective effect of A and O combinations against DM.(23).

In addition, anti-sera. Statistical analyzes were carried out using blood group tests to identify the number of blood groups. There are many studies that show blood types of type O + among diabetes, including females and males. /+ O/AB+/O-/B+/ AB blood types were found among them: DM1 = 16 sugar and DM2 = 14 type, since O- is less common among the blood groups of the diabetes group, and blood type 1DM is of the first type, and ABO It was found that in 30 patients with diabetes, the most common type was O+, and the least type was O-, and the number was equal between AB+/AB (24).

3.9. Relationship between Rheumatoid factor and DM Patients

Out of 30(100%) diabetes mellitus patients, all the cases in the present study show negative results for rheumatoid factor and there is no correlation between RF and DM, while other study presented a total of 44 diabetic patients who tested positive for RF; 16 of these patients were males (36.4%) and 28 were girls (63.6%). However, there are notable disparities (P=0.01) in the number and percentage of RF-positive people among healthy control participants; there are only 3 (4.3%) males and 2 (66.66%) females (25).

3.10. Relationship between cardiac problems and DM Patients

Out of 30(100%) diabetes mellitus patients, there were 2(7%) have a cardiac problem while the other 28(93%) DM patients don't suffer from any cardiac problem.

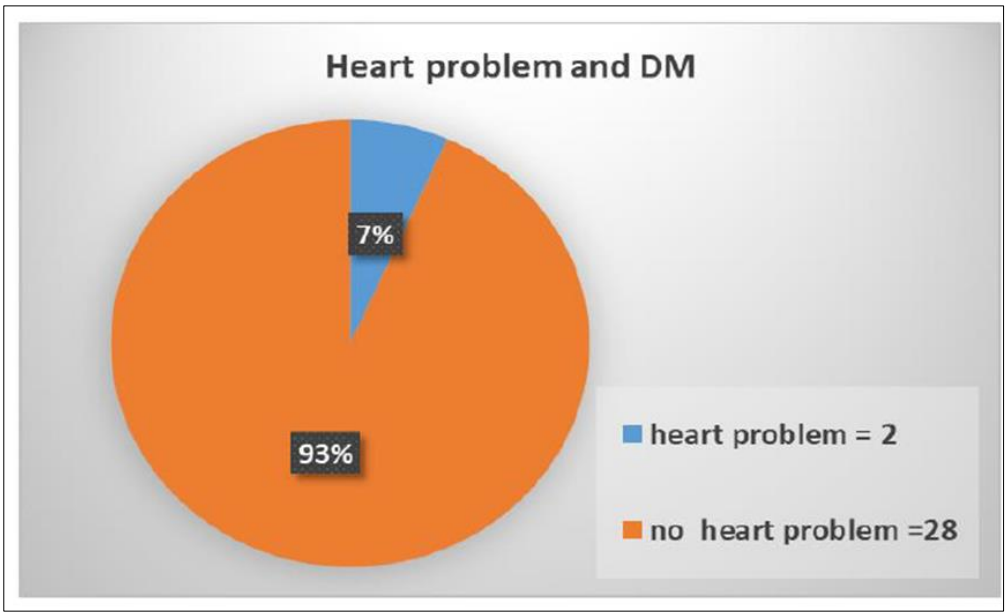

Figure 4 Distribution of cardiac problems in DM cases

The present study agreed to some extent with the majority of results were negative, and 1% were positive that explain in figure 4. Even if there is epidemiological evidence that shows a correlation between diabetes and heart failure, the existence of cardiomyopathy exacerbates the condition (26).

On the other hand, most the research articles shown adverse effect of DM on heart to show most these articles a positive relationship, Heart failure is more common in people with diabetes, and this risk is higher even when blood glucose levels are not yet high enough to be classified as diabetes (27).

3.11. Relationship between COVID-19 and DM Type-1 Patients

Out of 30(100%) diabetes mellitus patients, there were 15(50%) have positive COVID-19, 12(40%) has DM type 1 and 3(10%) has DM type-2 which appear in figure 5.

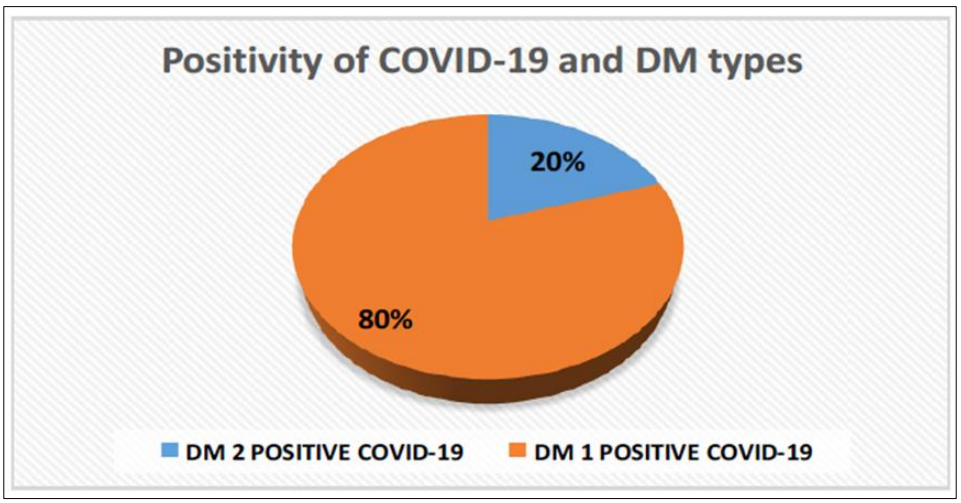

Figure 5 Distribution of COVID-19 positivity in DM cases

Diabetes mellitus and COVID-19 were found to be associated in a study that included both infected and healthy participants. During the COVID-19 pandemic, patients with diabetes mellitus should be mindful that the virus can raise blood glucose levels; hence, they should adhere more rigorously to the clinical guidelines for the management of diabetes mellitus, as outlined here (28).

Here is some broad advice for patients and doctors: pay close attention to when and how you take your insulin injections, and monitor your blood sugar levels more often than before. An individual should seek medical attention from a physician if their blood glucose levels are consistently above (29). Medical professionals treating diabetes mellitus should stress the importance of regular exercise and a balanced diet more heavily in view of the present international quarantine legislation (30).

4. Conclusion

- The risk factors of DM chronic complications seemed to be related to several parameters in a case-control study
- The present study concluded an appositive relationship between age, HbA1c, C-peptide, RBS, ABO blood groups and developments of diabetes mellitus.
- O+ is the most common ABO blood type, followed by A+, B+, AB+, AB-, and O-in terms of the association between ABO blood types and glucose intolerance instances.
- C-peptide significantly correlates with insulin secretion in DM
- The 2019 coronavirus illness (COVID-19) is more likely to occur and more severe in people with type 1 and type 2 diabetes metabolic syndrome.
- All the cases in the present study show negative results for rheumatoid factor and there is no correlation between RF and DM.

Recommendation

- To ensure that other diabetes patients and their caregivers have access to sufficient information regarding DM, we are developing educational programs.
- Make healthy eating and physical activity part of your daily routine.
- Keep your weight in a healthy range.
- Make sure to keep an eye on your blood sugar levels.
- Researchers should keep looking for more information on the health and socio-demographic characteristics of diabetics, as well as the effects of these factors on diabetes, by studying big samples of people with the disease.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

All authors had 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Statement of ethical approval

Ethical committees, patients, and their supporters must all provide their stamp of approval before any medical procedures may begin.

Statement of informed consent

Additionally, prior to sample collection, all individuals were informed and given the necessary consent to conduct the research and publish the results (Ethics The human study was approved by the Al-Qasim Green University, Babylon Province, Iraq Review Board).

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