Effect of metformin treatment on hematological parameters, HOMA-IR in patients with type 2 diabetes mellitus

Ruwaidah Hussein Abbas[1], Hameed Hussein Ali[2], Abid Ali Thaker[3]
[1,3]Department of Biology, College of Science, University of Anbar, Anbar, Iraq.
[2]Department of Chemistry, College of Science, University of Anbar, Anbar, Iraq.
Email: ruowidah39@gmail.com

Abstract
Aim: To assess BMI and the serum of HbA1c, Creatinine, HOMA-IR and QUICKI and hematological parameters in patients with T2DM were taking metformin in Iraq. Methodology: this is a cross-section study using case series of 80 patients with T2DM coming to the Baghdad Teaching Hospital Medical city, between December 2021 and April 2022. The blood sample was drawn to measure HbA1c, Creatinine, HOMA-IR, QUICKI and hematological parameters. The statistical analysis was conducted using the SPSS statistical package to analyze date. Results: According to the findings, there was a significant difference between the control group and T2DM patients undergoing metformin therapy in terms of blood levels of Creatinine, HOMA-IR and QUICKI and RBC except hematological parameters were non-significant. Conclusion: Body Mass Index, chemical parameters (HbA1c, Creatinine, HOMA-IR, QUICKI) and RBC were affecte to patients receiving metformin treatment for type 2 diabetes but regardinghematological parameters were non-affected compared with control group
Keywords: HbA1c, Creatinine, HOMA-IR, QUICKI, hematological parameters, Diabetic Mellitus, Metformi

1. Introduction

The World Health Organization (WHO) defines diabetes as a "metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both" (Balaji et al., 2019).
Type 2 diabetes (T2D) is the most prevalent form of diabetes, accounting for around 90% of all cases, with type 1 diabetes (T1D) accounting for the majority of the remaining 10% of cases (Pacaud et al., 2016).
Metformin is recommended by the International Diabetes Foundation as an initial treatment for type 2 diabetes in its 2005 publication of guidelines, The World Health Organization included metformin to its list of necessary drugs in 2011, over 50 years after its rediscovery. These trials' findings continue to support metformin as a treatment that is safe and efficient for the vast majority of patients, notwithstanding concerns surrounding the risk of metformin-induced lactic acidosis in individuals with concomitant conditions such congestive heart failure or hepatic and renal dysfunction. In reality, there is no longer a restriction on using metformin in people with poor renal function (Buse et al., 2016).
However, it has been demonstrated that metformin improves glycemic control via a number of ways. One of metformin's mechanisms of action is the non-competitive inhibition of the mitochondrial glycerophosphate dehydrogenase enzyme, which reduces intestinal glucose absorption, delays hepatic gluconeogenesis, and increases tissue glucose uptake, which lowers the rate at which lactate and glycerol are converted to glucose, this enzyme's inhibition lowers the amount of hepatic gluconeogenesis (Rena et al., 2017).
Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) it was discovered in the 1980s that your fasting glucose and fasting insulin levels might be used to determine how much insulin your pancreas needed to produce to control your blood sugar levels. Even though it may not be the best model overall, the most frequently utilized model in clinical research is this one (Gubin *et al.*, 2017).
Measuring insulin resistance directly is impractical, difficult, and infrequently used by clinicians. Other newly established indirect measures of insulin resistance include HOMA2, Quantitative Insulin-sensitivity Check Index (QUICKI), and the triglyceride/HDL ratio (the method is called hyperinsulinemic-euglycemic glucose clamp) (Gregory *et al.*, 2019).
Interestingly, Harita et al. (2009) postulated that type 2 diabetes risk is enhanced when blood creatinine is decreased, it might indicate a less amount of skeletal muscle. A decrease in the amount of skeletal muscle would mean fewer sites for insulin to target, which would promote insulin resistance because skeletal muscle is a significant target tissue for insulin, this leads to the development of T2D (Mohammed et al., 2014). The development of type 2 diabetes and reduced blood creatinine levels may be partially explained by this.
Hematological changes occur in T2DM patients' red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs), and coagulation systems. These changes affect the shape, metabolism, and function of these blood components (Arkew et al., 2021).

2. Materials and Methods

The study involved 80 people with Type 2 Diabetes Mellitus T2DM, 40 of whom were men and 40 of whom were women, who took metformin and were split into two groups: Group 1 (G1): included 40 T2DM patients taking ≤1000 mg\day for three years or more. Group 2 (G2): included 40 T2DM patients taking≥1500mg\day for three years or more. This study was conducted at the Baghdad Teaching Hospital in Medical city in the period from December 2021 to March 2022. Eighty of the T2DM patients were taking metformin after the clinical examination was done by the consultant physicians. After

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receiving the patients' consent and gathering the samples, their medical history and way of life were taken into consideration. after the consultation doctors' clinical diagnosis. Patients receiving insulin dosages were excluded, and T2DM was identified using the guidelines suggested by (Adler etal.,2021).

After a 12-hour fast, type 2 diabetes patients of health clinics had their blood samples taken in the morning. All participants gave a blood sample of 10 ml, which we split into two portions. 3 ml and 7 ml. The first part (whole blood) was dispensed into an EDTA tube for HbA1c and CBC determination on the same day, then the second portion was administered into a plain tube (tubes without heparin) and allowed to coagulate for 15 to 30 minutes at a temperature of 23 to 27 °C, The serum was separated into two portions and centrifuged at 3000 x g for 10 min to separate it; the first component was used to estimate creatinine for a biochemical experiment.

A mathematical equation developed by Matthews et al. was used to calculate HOMA-IR, and it may be simplified to HOMA-IR = FSI (micro units per milliliter) × glucose (FSG) ÷ 405, where FSI is fasting serum insulin and FSG is fasting Serum glucose (mg/dL).

Quicki Can be used to calculate of fasting plasma glucose (mg\dl) and fast insulin (uU\mL)

QUICKI = 1\(logI + logG)

Finally, calculate by an equation (Adler et al.,2021).

BMI = weight (kg) / height (m²)

3. Results

In this study, out of 120 sample size, 80 subjects were used metformin dose (≤1000 mg\day and ≥ 1500 mg\day) and 40 subjects were healthy control. The means ± S.E of (BMI, HbA1c, Creatinine, HOMA-IR and QUICKI) for healthy subjects and patients with diabetes treated with metformin ≤ 1000 and ≥1500 mg per day (24.35±0.45 vs. 27.49±0.58, 28.03±0.53 Kg/m2), (5.23±0.10 vs10.03±0.38, 9.00±0.37 %), (0.66±0.02 vs 0.77±0.041, 0.88±0.039 mg\dl), (1.32±0.05 vs 4.67±0.34, 5.87±0.49), (0.36±0.0040 vs 0.31±0.0033, 0.30±0.0035) respectively, and all this parameters were highly significant (P<0.001) increment between healthy subjects and patients with diabetes treated with metformin. In addition to hematological parameters the means ± S.E of (RBC, HGB, HCT, MCV, MCH and MCHC) (4.10±0.08 vs 4.53±0.12, 74.10±0.08 x10*12\L), (12.44±0.17 vs 12.30±0.20, 12.28±0.22 g\dl, respectively), (37.35±0.64 vs 38.11±0.81, 37.75±0.79 %), (88.14±1.21 vs 87.19±0.81, 85.71±1.39 fL), (29.86±0.61 vs 29.25±0.49, 28.50±0.70 pg) and (33.83±0.40 vs 33.53±0.32, 33.17±0.39 g\dl) respectively While all parameters are not significant (P < 0.05) except for RBC showed significant (P<0.05) increment as shown in the table 1.

In the table 2 and figure 1 (A – k), the ROC curve demonstrated that the (HOMA-IR and HbA1C) levels have exhibited an excellent method for discriminating between control and patients with DM [AUC=0.996, S.E=0.003, 95%CL=0.990 to 1.000 and p≤ 0.0001],[AUC=0.990, S.E=0.010, 95%CL=0.970 to 1.000 and p≤ 0.0001] respectively, with a value of [AUC=0.786, S.E=0.045, 95%CL=0.697to 0.875 and p≤ 0.0001] BMI is considered as a highly significant parameter for disease risk. Moreover, this study showed the values of (Creatinine, RBC and HCT) parameters have been appeared as significant diagnostic tool between control and sick inndividuals [AUC=0.689, S.E=0.048 , 95%CL=0.596 to 0.783 and p=0.001],[AUC=0.712 ,S.E=0.049 ,95%CL=0.617 to 0.807 and p=0.0001] and [AUC=0.527 ,S.E=0.056 ,95%CL=0.417 to 0.636 and p=0.636] respectively.

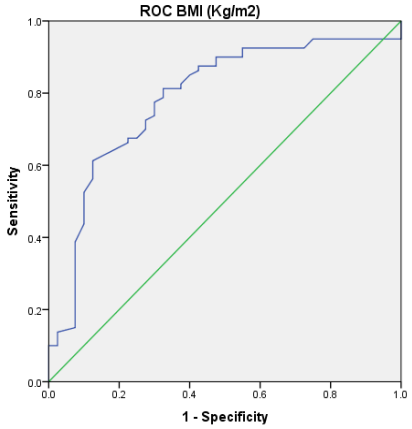
Finally, age, HGB, MCV, MCH, MCHC and QUICKI showed low validity in predicting of DM with AUC and S.E [AUC=0.580, S.E=0.057,95%CL=0.469 to 0.692andP=0.152],[AUC=0.499,S.E=0.057,95%CL=0.387to0.612andP=0.991]],[AUC=0.403,S.E=0.060,95%CL=0.285to0.521andP=0.085],[AUC=0.399,S.E=0.059,95%CL=0.284to0.513andP=0.071],[AUC=0.466,S.E=0.059,95%CL=0.351to0.581andP=0.542]and[AUC=0.049,S.E=0.026,95%CL=0.000to0.100andP=0.0001].

Table (1) Comparisons of parameters for the DM groups and healthy group.

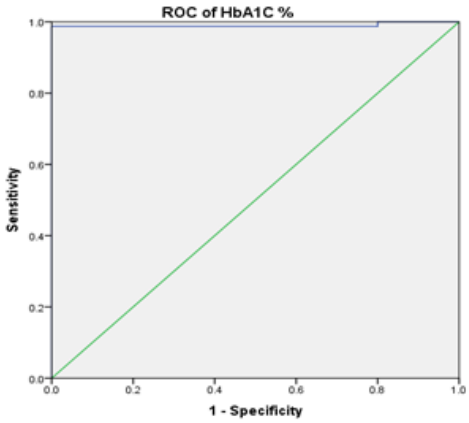
Parameters	Patients Groups		Control	P-Value
	Mean ±SE Group 1 NO=40	Mean ±SE Group 2 No=40	Mean ±SE No=40	
BMI (kg/m2)	27.49±0.58	28.03±0.53	24.35±0.45	0.0001
HbA1c (%)	10.03±0.38	9.00±0.37	5.23±0.10	0.0001
Creatinine (mg\dl)	0.77±0.041	0.88±0.039	0.66±0.02	0.0001
HOMA-IR	4.67±0.34	5.87±0.49	1.32±0.05	0.0001
QUICKI	0.31±0.0033	0.30±0.0035	0.36±0.0040	0.0001
RBC x10*12\L	4.53±0.12	4.60±0.12	4.10±0.08	0.002
HGB (g\dl)	12.30±0.20	12.28±0.22	12.44±0.17	0.828
HCT %	38.11±0.81	37.75±0.79	37.35±0.64	0.773
MCV fL	87.19±0.81	85.71±1.39	88.14±1.21	0.334
MCH pg	29.25±0.49	28.50±0.70	29.86±0.61	0.283
MCHC (g\dl)	33.53±0.32	33.17±0.39	33.83±0.40	0.458

Group 1= (Patient ≤ 1000 mg. day), Group 2= (Patient≥ 1500 mg. day)

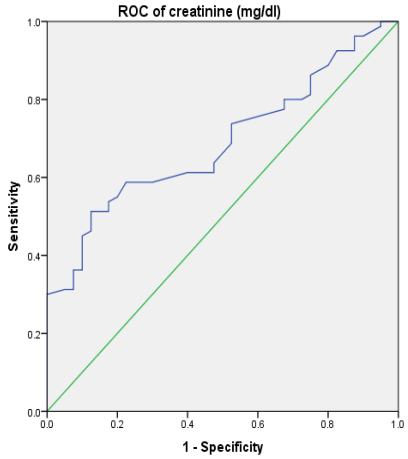
Table 2: Area under the ROC curve for all analyzed biomarkers.				
Parameter	AUC	S.E	95% Confidence Interval	P-value
BMI (kg/m2)	0.786	0.045	0.697 to 0.875	0.0001
HbA1c (%)	0.990	0.010	0.970 to 1.000	0.0001
Creatinine (mg\dl)	0.689	0.048	0.596 to 0.783	0.001
HOMA-IR	0.996	0.003	0.990 to 1.000	0.0001
QUICKI	0.049	0.026	0.000 to 0.100	0.0001
RBC x10*12\L	0.712	0.049	0.617 to 0.807	0.0001
HGB (g\dl)	0.499	0.057	0.387 to 0.612	0.991
HCT %	0.527	0.056	0.417 to 0.636	0.636
MCV fL	0.403	0.060	0.285 to 0.521	0.085
MCH pg	0.399	0.059	0.284 to 0.513	0.071
MCHC g\dl	0.466	0.059	0.351to 0.581	0.542



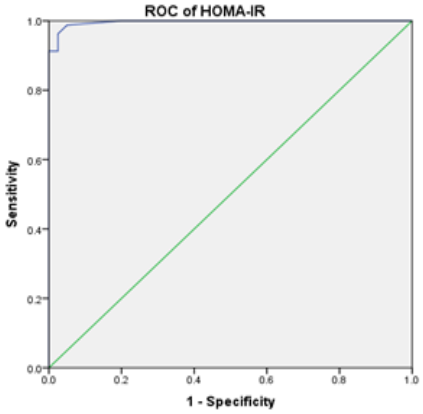
(A) ROC of BMI



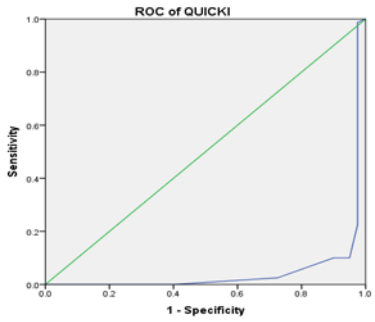
(B) ROC of HbA1c



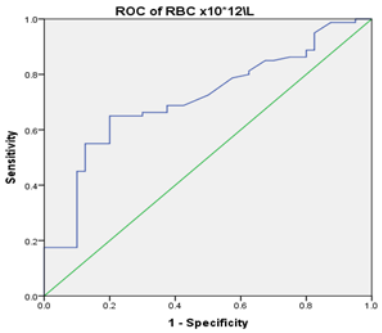
(C)ROC of Creatinine



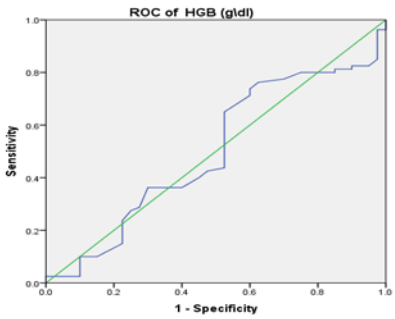
(D)ROC of HOMA-IR



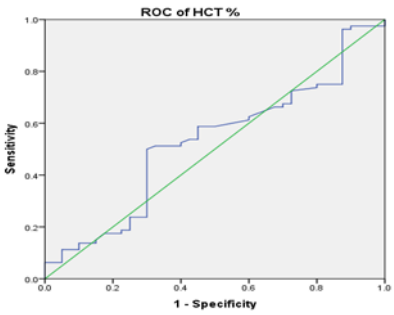
(E) ROC of Quicki



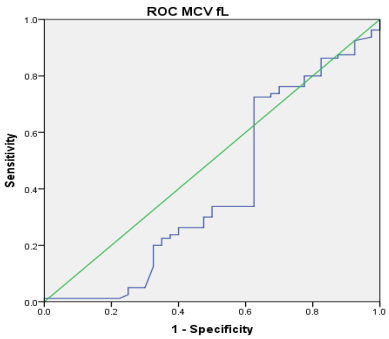
(F) ROC of RBC



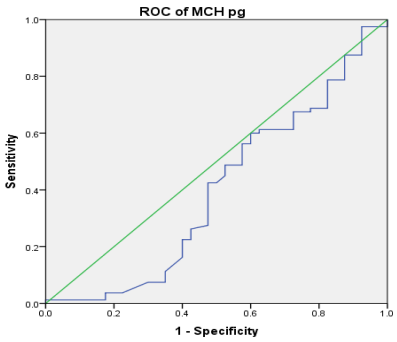
(G) ROC of HGB

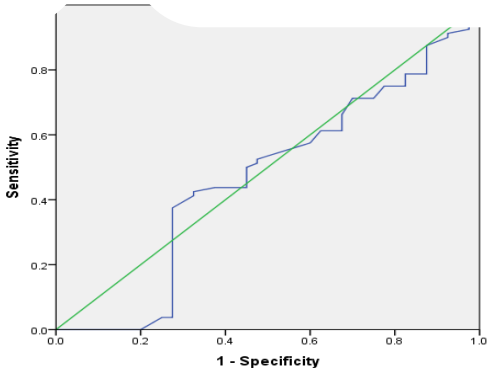


(H) ROC of HCT



(J)





(S) ROC of MCHC
(K) ROC of MCHC

Figure 1 (A – k): Curves of Receiver Operating Characteristics displaying the AUC between the examined parameters' sensitivity and specificity

4. Discussion

A recent systematic review found that metformin treatment is associated with BMI reduction in and adolescents in comparison with placebo, a study by Oza to show the long-term therapeutic benefit of metformin therapy in type 2 diabetes mellitus patients who are not obese (BMI 25 kg/m2). HbA1c levels were significantly lowered by metformin in non-obese subjects (Oza, 2017).

Despite the fact that several studies have demonstrated metformin's benefits for those with non-obese diabetes, the observation times were just briefly. reported that a

prospective study conducted over a 12-month period found no difference in the reduction of HbA1c by metformin between 303 Japanese people with type 2 diabetes who were neither obese nor non-obese (BMI 25 kg/m2) and 300 who were. (Williams et al.,2014).

After starting metformin for type 2 diabetes mellitus, Ito et al. (2010) retrospectively showed a significant decrease in HbA1c levels of 0.79 percent in 58 individuals with a BMI of 22 kg/m2, 0.81 percent in 81 patients with a BMI of 22 kg/m2, and 0.73 percent in 136 patients with a BMI of 25 kg/m2.

Reviewed by Brufani et al., (2013) was eleven trials. Metformin was given daily doses of 1,000–2,000 mg for 6–12 months, as compared to placebo or lifestyle change alone, lowering BMI by 1.1–2.7. Additionally, after using metformin, fasting insulin resistance improved. In one investigation, post-treatment follow-up was done, demonstrating that the reduction in BMI vanishes a year after treatment is stopped.

In five out of ten investigations, metformin was found to significantly lower HbA1C, which in turn improved glycemic control (CDC,2017), this agreement with our study.

Metformin decreases insulin-dependent hepatic gluconeogenesis while improving peripheral glucose absorption and utilization. The effects of various metformin dosages (1000 and 1500 mg) on the HbA1c levels in T2DM patients were examined in the findings of the current investigation. The average HbA1c levels in treated groups of diabetes individuals are consistent with other findings (Chiang et al.,2018).

But in comparison to baseline readings, the percent drop in HbA1c brought about by the administration of various metformin dosages was (-10%, -18.3%, and -28.2%), respectively; This conclusion is consistent with findings from other earlier research, and a short treatment period may have had an impact on the outcomes. HbA1c baseline values in the treated groups were lower than fasting glucose levels at zero time, which may have indicated that the HbA1c values were not in steady state at the beginning of treatment. (Rena etal.,2017)

Due to the possibility of the potentially fatal side effect, lactic acidosis, Men and women with blood creatinine levels of 1.5 mg/dL or higher and 1.4 mg/dL or higher, respectively, should not use metformin. Metformin-associated lactic acidosis is also more likely to occur in the presence of risk factors such severe dehydration (which reduces tissue perfusion), old age, severe alcohol usage, hepatic impairment, sepsis, shock, hypoxia, and congestive heart failure. (Hsu et al., 2018).

Since the kidneys excrete metformin mostly unaltered, renal dysfunction may result in metformin buildup and a higher metformin concentration, which has been postulated to induce lactic acidosis (Hsu et al., 2018)

A reliable clinical and epidemiological technique for describing the pathogenesis of diabetes is HOMA-IR. In addition, compared to the euglycemic clamp test, it is a simple, safe, less intrusive, and less costly approach, and its outcomes are well associated with those of the latter (Wongwananuruk et al.,2012) (Maric etal.,2019). Numerous epidemiologic research have used HOMA-IR to measure participants' IR because of its strengths (Qu etal.,2011). It is a well-known fact that type 2 DM arises from IR and is linked to aberrant metabolic processes. (Lebovitz etal.,2001). The levels of HOMA-IR can also be impacted by diabetic drugs including metformin, glimepiride, and SGLT2 inhibitors (So, A. et al.,2020). This was noted in the results of the study.

A brand-new mathematical translation of fasting blood sugar and insulin levels is called the quantitative insulin-sensitivity check index (QUICKI). In comparison to minimal-model estimates, QUICKI greatly improves the linear correlation between glucose clamp measurements of insulin sensitivity in obese and diabetic participants (Badawi et al.,2022).

Analysis of quantitative insulin sensitivity check index (QUICKI) suggests that metformin treatment increased insulin sensitivity (Zhu et al.,2022), this is agreement with our study.

A non-enzymatic glycosylation of Hgb and RBC membrane proteins and increased ROS generation due to prolonged hyperglycemia may be the reason of the lower RBC count and result in impaired deformability, RBCs age more quickly and aggregate more often. It has also been demonstrated that these RBC alterations significantly raise blood viscosity, which negatively impacts microcirculation in diabetes and results in microangiopathy (Awofisoye et al., 2019)

In people with diabetes RBCs appear to be submerged in a "glucose bath" when glucose is not used up in blood circulation, changing their biconcave "ring-like" shape into a flat ring and eventually a convex balloon. Due to its tiny size, glucose can readily pass through the erythrocyte membrane and cause internal swelling. RBCs are typically 10-15% larger in diameter, hence diabetes patients have 53% more RBCs per cubic millimeter of blood. (In comparison to the normal control, each diabetic RBC's volume increases by 53% (1.15 1.15 1.15) when its radius increases by 15%.)

The increased viscosity of blood in diabetes individuals may be due to this in particular. Additionally, these RBCs appear to have a propensity to adhere to one another, which may be the cause of the accelerated coagulation linked to DM. RBCs have increased friction both within themselves and when moving through microvessels as a result of their restricted mobility and grouping together (Ghosh et al.,2016). This is consistent with our results, where we found elevated erythrocytes in patients.

In contrast to our findings, studies from Pakistan and Gondar, northwest Ethiopia, discovered that T2DM patients had higher RBC counts and Hgb concentrations than controls. The impact of insulin resistance may be to blame for this, which increases RBC count, levels of Hgb, and HCT and is associated with activation of erythroid progenitors (Kim et al., 2017)

Inflammation is significantly influenced by platelet activation, and atherothrombosis causes CVD in patients with T2DM. Accelerated atherosclerosis exacerbates diabetes. In diabetics with coronary heart disease, nephropathy, and retinopathy, MPV has been found to be increased. A change in platelet stimulation or the rate of platelet production is indicated by variations in mean platelet volume (MPV) (Korniluk et al., 2019).

5. Conclusion

A highly significant relationship was found for HbA1c, creatinine, HOMA-IR, QUICKI and RBC parameters for patients with type 2 diabetes treated with metformin compared to healthy controls, except that hematological parameters did not show any significant relationship.

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