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DOKTORI ISKOLA

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DLOVAN ALI JALA

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című program

Programvezető: Dr. Vásárhelyi Barna, egyetemi tanár

tanárTémavezető: Dr. Gyarmati Béla, egyetemi docens

EXPLORING LABORATORY PARAMETERS THAT IMPACT HBA1C LEVELS

PhD thesis

Dlovan Ali Jalal

Semmelweis University Doctoral School

Theoretical and Translational Medicine Division

Supervisor: Béla Gyarmati, MD, PhD

Official reviewers: Domokos Máthé, MD, PhD
Béla Köves MD, PhD

Head of the Complex Examination Committee: Rozgonyi Ferenc Ph.D

Members of the Complex Examination Committee: Karvaly Gellért Ph.D
Bhattoa Harjit Ph.D

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List of abbreviations

AC – affinity chromatography (AC)

AHA – American Heart Association

ALT – alanine aminotransferase

CBC – complete blood count

CE – capillary electrophoresis

CGM – Continuous glucose monitoring

CRP – C-reactive protein

CVD – cardiovascular disease

GDM – gestational diabetes mellitus

Hb – haemoglobin

HbCarb – carbamylated hemoglobin

HbF – fetal hemoglobin

HbA1c – glycated hemoglobin

HDL – high density lipoprotein

IEC- Ion exchange chromatography

IFCC – International Federation of Clinical Chemistry

IA – immunoassay

IL-1 – interleukin-1

IL-6 – interleukin-6

LDL – low density lipoprotein

LMICs – lower middle income countries

RBC – red blood cell

RDW - Red blood cell distribution width

RMP – Reference Measurement Procedure

sdLDL – small dense LDL

T2DM – type 2 diabetes mellitus

TC – total cholesterol

TG – triglyceride

TNF – tumor necrosis factor

VLDL – very low density lipoprotein

1. Introduction

1.1 Generation of glycated hemoglobin

The chemical binding of Hemoglobin (Hb) with a sugar molecule generates glycated hemoglobin (HbA1c, glycohemoglobin) (Kilpatrick ES, 2008). When present in the bloodstream, the majority of mono-sugars, including glucose, galactose, and fructose, spontaneously (i.e., non-enzymatically) bind with hemoglobin (McPherson JD, 1988).

Exposure to higher levels of blood glucose lead to higher levels of glycated Hb, frequently a sign of abnormal glucose control. Glycation is the process by which sugars attach to hemoglobin, and the reference standard is based on HbA1c, which is defined as a component of Beta-N-1-deoxy fructosyl hemoglobin (Pongudom, 2019).

The name HbA1c comes from the cation exchange chromatography separation of haemoglobin type A. The first part to separate, was given the name HbA0. The subsequent fractions were given the names HbA1a, HbA1b, and HbA1c, according to the order in which they were eluted. (Miedema, 2005).

The HbA1 fraction, which makes up around 6% of all HbA in normal individuals, is divided into HbA1a1, and three other fractions, HbA1a2, to HbA1b, and HbA1c fractions based on their chromatographic and electrophoretic characteristics. HbA1c, the most common of these fractions, accounts for around 5% of the total HbA fraction in healthy people. However, the quantity of glycated hemoglobin in the plasma rises along with the average plasma glucose. This phenomenon is the basis of the assessment of glucose control exposure during the previous 2-3 months (Sherwani SI, 2016).

1.2 Short history of the discovery glycated hemoglobin

Huisman et al. (Huisman TH, 1958) initially isolated HbA1c in 1958, then Bookchin and Gallop (Bookchin RM, 1968) first identified it as a glycoprotein in 1968. Rahbar et al.'s study on the higher HbA1c values in diabetic patients was published in 1969 (Rahbar S, 1969). In 1975, Bunn et al. (Bunn HF, 1975) discovered the pathway by which HbA1c is formed. Koenig et al.'s (Koenig RJ, 1976) initial suggestion that the HbA1c be used as a biomarker for tracking glucose levels in diabetic patients was made in 1976.

1.3 Detection of glycated hemoglobin

HbA1c is primarily evaluated to estimate mean of blood sugar level during the past three months, but recent American guidelines also recommend it to be used to diagnose

diabetes mellitus (Sherwani SI, 2016). Since the average lifetime of RBCs is normally 3-4 months, the test is representing this three-month period (Giacco F, 2010).

Diabetes Control and Complications Trial units (Lind M, 2021) According to the recommendations of the International Federation of Clinical Chemistry (IFCC), several other countries in Europe and in Asia, including England, and some other countries, express HbA1c results as millimoles per mole (Aldasouqi SA, 2008). However, in clinical practice mmol/mol units are converted to per cent values for clinical decision making. The NGSP, therefore, continues to advise using %s with one decimal place for units. Corresponding decision limits are reported dominantly as % units. (Eyth E, 2023).

1.4 The Range of HbA1c

The typical range for non-diabetics is 4.0%-5.6% HbA1c. pre-diabetic status is assumed if HbA1c reading in range of 5.7-6.4 %s whereas measurement above 6.4 % is more related to fairly uncontrolled DM. The suggestions for people with diabetes include leading a healthy lifestyle (diet and exercise) and keeping their HbA1c levels under 7.0%. HbA1c levels and diabetes complications are correlated; as levels rise, so does the chance of developing these issues (Cohen RM, 2010).

In some specific conditions HbA1c levels may be altered independently of glucose levels. Extremely low hemoglobin levels or shorter RBC life (glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, etc.) may provide an erroneous low result (Sherwani SI, 2016). Atypically high concentrations of HbA1c can also be caused by excessive vitamin C, B, and E supplementation, elevated cholesterol levels, liver, and renal illness, and other conditions (Dakhale GN, 2011).

1.5 Methods for HbA1c Analysis

There are two primary analytical concepts: the first is based on the physio-chemical characteristics of Hb fractions (separation techniques), and the second is based on immunoassay (Fig. 1.2). These methods measure a variety of analytes, however by applying the Reference Measurement Procedure (RMP) of the International Federation of Clinical Chemistry (IFCC), they can standardize tests (Weykamp, 2013).

1) Methods for fraction separation: Non-glycated Hb and HbA1c have different chemical properties. This concept is utilized by affinity chromatography (AC), capillary electrophoresis (CE), and ion exchange chromatography (IEC).

IEC: The pI of HbA1c and Hb differs by 0.02 units. This distinction is sufficient for HPLC to differentiate HbA1c from nonglycated Hb. IEC can detect sickle cell hemoglobin (HbS), minor fast hemoglobin (HbA1a/b), carbamylated hemoglobin (HbCarb), and fetal hemoglobin (HbF) (Nasir NM, 2010).

CE: The charge difference between HbA1c and other Hb fractions is used in this approach. High-voltage electrical field and electroosmotic flow are used to separate materials.

AC: Glycated Hb molecules exhibit an affinity for boronic acid, and HbA1c is kept on the column, but non-glycated Hb freely passes through a column filled with boronic acid-coated particles. Approximately 15 different lysine residues in Hb are the sites where glucose could bind to, in addition to the Nterminal valine of the -chain (HbA1c). (Weykamp, 2013).

2) Chemical methods: HbA1c concentration can be also assessed in chemical testing is determined by a particular chemical reaction to the glycated N-terminal valine of the -chain. Photometry is used to measure the total Hb concentration.

Immunoassays (IA): IA involves adding an excessive amount of anti-HbA1c antibodies to a hemolyzed sample. The extra antibodies bind to HbA1c and agglutinate. Using a turbidimeter or nephelometer, the turbidity of the resultant immunocomplexes is determined photometrically. In the pre-incubation period, the total Hb concentration is also bichromatically assessed (Rose AM, 1995).

1.6 Clinical Significance of glycated hemoglobin

Blood loss, hemolytic anemia, and hypersplenism are common causes of alterations in HbA1c readings (Radin MS, 2014); for example the acute blood loss causes transient decrease in HbA1c levels.

The A1C-Derived Average Glucose (ADAG) study established the link between average glucose levels and the hemoglobin A1C across communities and kinds of diabetes, laying the groundwork for its clinical use in the 1980s (WHO, 2011).

In 2009, a panel of international experts recommended using HbA1c as an aid to diagnose diabetes and set a cut-off of 6.5%. Confirmation necessitates a second HbA1c test unless clinical symptoms and plasma glucose levels are greater than 11.1 mmol/l (200 mg/dl) are present. A suggested high-risk range of 5.7-6.4% is indicated by

readings that are just under 6.5% and indicate intermediate hyperglycemia (Committee., 2009).

To properly titrate medicine and improve outcomes for individuals with diabetes, monitoring glycaemic control on a regular basis can be difficult in LMICs. The preferred test for determining drug titration and monitoring glucose control in high-income nations is HbA1c (glycosylated hemoglobin), which is frequently accompanied by home glucose capillary or interstitial glucose monitoring (Niwaha AJ, 2021).

1.7 Relation between HbA1c and lipid profile

Dyslipidemic profiles can appear prior to the onset of type 2 diabetes and are independent risk factors for the condition. Dyslipidemia can also decrease pancreatic beta-cell survival and activity, especially in diabetics (Bardini G, 2012).

In diabetic patients, the HbA1c value and lipid profile have obvious correlation. Along with a reliable glycemic index, HbA1c can be used to predict dyslipidemia, hence treating dyslipidemia early in T2DM patients will help them avoid developing cardiovascular disease (CVD) (Hussain A, 2017). The severity of dyslipidaemia increases in people with higher HbA1c values, and there is a positive link between HbA1c level and TC and TG in diabetic individuals (Khan, 2007).

Patients with T2DM frequently have increased plasma levels of TG, total Cholesterol [use the abbreviations consequently, e.g. TC, and LDL-C, but low levels of HDL-C (Omodanisi EI, 2020). Dyslipidaemia, which was brought on by the addition of rising cholesterol levels, served as the main catalyst for the development of atherosclerosis independently of hyperglycemia (Nnakenyi ID, 2022).

1.8 Hyperglycaemia and C-reactive protein

Low-grade inflammation characterized by moderately increased CRP levels is a risk factor for atherosclerosis. This low-grade inflammation is detected by high sensitivity-CRP (hs-CRP) tests and is usually hallmarked by CRP levels between 0.5 and 10 mg/L (Sproston NR, 2018). Chronic increases in CRP may affect the biological functions of endothelial function, coagulation, fibrinolysis, and low-density lipoprotein (LDL) oxidation, as well as the stability of atherosclerotic plaques (WP, 2010). The CRP amount is also associated to the rate of cardiovascular event-free survival (Halcox JP, 2014).

The increase in cardiovascular risk is accompanied by the concentration-dependent rise in HbA1c levels. Studies have shown that subgroups follow the same pattern. Fasting glucose levels above and below particular thresholds are also associated with an increased risk of a cardiovascular incident (Pfützner A, 2010).

1.9 Hyperglycaemia and uric acid

The end-product of purine metabolism in the bloodstream is uric acid. Increased risk for cardiovascular disease and metabolic illnesses including the metabolic syndrome and diabetes mellitus are linked to elevated serum uric acid levels (Gherghina M-E, 2022). Hyperuricemia patients have a much higher risk of developing diabetes. Because uric acid and glucose metabolism are related, some research suggests that uric acid may be linked to abnormalities of the glycometabolic system. The relationship between uric acid and blood glucose levels is not linear, though. Patients with type 2 diabetes mellitus who have hyperuricemia have a higher chance of developing diabetic nephropathy. Patients with diabetes mellitus type 2 may have higher serum uric acid levels as macroalbuminuria and microvascular dysfunction progress. Uric acid levels rise with increasing blood glucose concentrations in the normal and prediabetes population (Xiong Q, 2019).

1.10 Association of HbA1c with age and gender

In the age ranges of 30-59 years, males had substantially higher HbA1c readings than females ($P < 0.05$). It is most likely because men in this age range have blood pressure and blood lipids that are more challenging to control, while women may be more susceptible to physiologic cycle effects. Gender differences were observed in the association between HbA1c and age, as in other publications. This finding might be explained by the earlier hypothesis that menstruation women had lower hemoglobin levels and faster erythrocyte turnover (Ma Q, 2016). Women had a steeper slope than men in the peri- and post-menopausal age groups. Humans have a diversity of changes as they age, including: i) the function of the pancreatic islets gradually diminishes; ii) tissue sensitivity to insulin and insulin receptor activity gradually drop; iii) the amount of muscle tissue gradually decreases and glucose uptake generally declines. The combined effect of these variables causes blood glucose to rise progressively with aging, which elevates HbA1c levels as well, especially as people age (Brzozowska M, 2020).

1.11 The relation between HbA1c and erythrocytes parameters

1.11.1 HbA1c and RBCs count

Thus a low level of HbA1c may be seen in conditions that shorten the RBCs lifespan. These observations showed that HbA1c is a very poor marker for diabetic patients with haemolytic anemia (Earl S Ford, 2011). On the other hand, a decrease in RBC turnover is followed by an increase in the glycation rate of Hb, which eventually results in a high HbA1c measurement (Zhenhe Huang, 2018). HbA1c levels are considerably lower in patients with iron deficiency anemia (IDA) than in those without IDA, and these levels rise once the IDA is treated (Kalairajan S, 2019).

1.11.2 HbA1c and red cell distribution width

The red cell distribution width (RDW), which is employed to distinguish between some kinds of anemia, evaluates the heterogeneity of the volume of RBCs. HbA1c and RDW were found to be significantly positively correlated in healthy non-diabetics and diabetics (Veeranna V Zalawadiya SK Panaich SS, 2012), (Bhutto AR, 2019). While Salimon et al. discovered in their study that men have demonstrated a substantial association of HbA1c with RDW in men when compared to women (Salimon AH, 2017). RDW and HbA1c have been demonstrated to positively correlate in another study by Sherif et al., however statistically, the association was not significant (Sherif H, 2013). In addition, Lippi et al.'s study showed a strong association between HbA1c and RDW even after adjusting for age and gender (Lippi G, 2014).

1.12 Vitamin D levels and HbA1c

There has been some global emphasis recently focused on the role of vitamin D in the genesis of diabetes. Numerous studies have been conducted on the additional benefits of vitamin D for the skeleton. The identification of 1,25 (OH)2D3 and 1-Alpha-Hydroylase expression in immune cells, pancreatic beta cells, and various other organs beyond the bone system supports the role of vitamin D in the development of type 1 and type 2 diabetes. Vitamin D deficiency appears to be linked to diabetes mellitus, metabolic syndrome, and a decreased HbA1c level. The majority of prospective observational and cross-sectional studies that were utilized to collect data on humans showed that the prevalence or incidence of high HBA1c in type 2 diabetes was inversely correlated with vitamin D levels (Asma Akhter, 2024), (Ghavam S, 2018), (Alqahtani RM, 2023).

2. Objectives:

I. Goal one: Prior research has suggested that the calculated HbA1c, which is derived from self-measured glucose and previous HbA1c values, should be utilized to evaluate glycaemic control in individuals with DM, especially those who have strong glycaemic control (Temsch W, 2008). Changes in HbA1c can occur regardless of the glycaemic management. Age, sex hormones, visceral fat distributions, physiologic and genetic factors, and socioeconomic position are some of the potential causes of these variances (Cohen RM, 2006).

Our work tested the possibility of developing a formula to describe the relationship between HbA1c levels and serum levels of lipid profile, uric acid, and C-reactive protein.

II. **Goal two:** The hemoglobin molecule undergoes structural and functional changes as a result of the hemoglobin protein's continuous glycation brought on by prolonged exposure to hyperglycemia in red blood cells (Turpin C, 2020). Hyperglycemia affects RBCs in a number of additional ways, including altered internal viscosity and mechanical features, increased aggregation, and osmotic fragility, which in turn affects erythrocyte structure and hemodynamic parameters (Li Q, 2018).

Our research investigated whether routinely requested red blood cell parameters should be considered in the interpretation of HbA1c levels in patients in general medical practice.

III. Goal three: A deficiency in vitamin D can contribute to deteriorating several illnesses and even may be a risk factor for developing DM (Pittas AG, 2012). Lower levels of 25(OH) D have been linked to a higher frequency of metabolic syndrome and diabetes mellitus (Kim YS, 2018).

This work analyzed the possible association between HbA1c and vitamin D.

IV. **Goal four:** It is well recognized that older adults are more likely to have type 2 DM, lower glucose tolerance, and impaired fasting glucose (MB, 1979). Some researchers have demonstrated that older individuals without diabetes have greater HbA1c levels (FQ, 1999), (Pani LN, 2008), (Roth J, 2016), (PS., 2007),

This work tested whether efforts to normalize HbA1c levels are impaired in patients with ages and genders.

3. Methods

3.1 Data extraction for Goal 1

Our laboratory database contains 200 million records of laboratory measurements from the period between 2008 and 2018. Records include the anonymized patient identifier, patient's age, gender, date of measurement, the name of the analyte, and the numeric result along with units. We selected those patients' data who presented for the first time for HbA1c measurement during this period and had also complete blood count (CBC), total cholesterol, LDL- and HDL, triglyceride, uric acid and CRP levels measured within 1 month. From this subgroup, patients with CRP levels above 10 mg/L were excluded. The studied cohort was further narrowed down to patients between 20 and 90 years of age to ensure sufficient number of individuals in each age group. As HbA1c levels are heavily influenced by severe anemia, we also omitted patients with haemoglobin values below 100 g/L from the analysis. Table (2.1) and Table (2.2) summarize the number of patient populations analysed.

In the analysed period, HbA1c measurement was performed with BioRad HPLC kits; clinical chemistry parameters were measured by manufacturers' kits on Roche and Beckman-Coulter clinical chemistry analysers (the reference values for parameters were comparable on different platforms; the lower level of detection (LOD) for CRP is 1 mg/ L); CBC was measured with Sysmex XN haematology analysers. Finally, data of 9599 male and 10,817 female patients were used for the analysis. **Statistical analysis of method for goal No. 1:** For parameters other than gender and age, the model used the log-transformed value of serum level, as the distribution of these values were closer to normal. In the model used for the ranking of variables based on their impact on HbA1c levels, the values were also rescaled using a z-score-like approach.

3.2 Data extraction for Goal 2

Our laboratory database was recorded between 2008 and 2018 contains laboratory measurement records. One the anonymized patient identifier, the patient's vital age, sex, date of measurement, name of analyte, numeric result and the unit of measure. The database regarding medical diagnosis and treatment data.

We have selected the data of patients who have for the period of HbA1c measurement and for RBCs indices within 1 week. We used a measured value for the first time within the examined period, so each patient's value was included in the study

just once. HbA1c levels were determined using a BioRad HPLC system (Hercules, CA, USA), the blood count was performed by Sysmex XN Hematology determined with an analyzer (Kobe, Japan). Table 1- summarizes the data sorting process. We used the data of more than 60,000 patients for analysis. **Statistical analysis of method for goal No. 2:** The relationship between individual parameters and the HbA1c level to estimate a z-score-like transformation and calculated the coefficient (coefficient of estimates - CE) value. After that, the HbA1c value and red blood cell parameters (HB, HCT, MCV, RBC, RDW) a multiple regression model was used to evaluate values obtained on the basis of regression analysis using the individual variables on HbA1c its effect was described with a summarizing formula for both genders case. Using this formula, we calculated that each with different RDW, MCV and HB values of variables, what HbA1c value is expected effective (estimated mean HbA1c value), and this was compared to with the mean value of measured HbA1c values. The calculations were performed using the R v4.1.2 program package (R Statistical Software v4.1.2; R Core Team 2021, Vienna, Austrasia) was carried out.

3.3 Data extraction for Goal No. 3

Records include the anonymized patient identifier, patient's age, gender, date of measurement, the name of the analyte, and the numeric result along with units. Data of 9599 male and 10817 female patients were used for the analysis. Data assembly was performed by capturing the results of HbA1c and serum vitamin D, from electronic health records of different patients visiting the Semmelweis hospital. The results represent the initial laboratory before the medical intervention. **Statistical analysis of method for goal No. 3:** The skewed data for vitamin D levels were returned to normality by applying a log transformation. The relationship between the level of vitamin D and the HbA1c was investigated using the Pearson correlation coefficient. To determine the correlation between the level of vitamin D and the HbA1c, multiple linear regression analysis was utilized. The results' statistical significance was represented by a confidence interval (CI) of 95% ($P<0.05$), with a predefined significance threshold of 5%.

3.4 Data extraction for Goal No. 4

The investigation employed data from 6069 males and 5998 females' participants' two visits' worth of HbA1c readings. The process of data assembly involved gathering

HbA1c results from various patients' electronic health records who visited the Semmelweis hospital. **Statistical analysis of method for goal No. 3:** The SPSS software program version 23.0 was used for data analysis. The descriptive (frequency, percentages, mean, and standard deviation) and inferential (Chi-square test) statistics were calculated according to the variables studied.

3.5 Ethical approval: The study was approved by the National Ethical Committee. The data collection was carried out by the Health Science approved by the Council of Ministers under TUKEB number 52331/2019.

Table (3. 1): Analysed patient population.

	men	**Women**
Patients' number in database	246,568	352,247
Patients' records with HbA1c value	92,841	100,645
Of those, patients with first time HbA1c value	33,031	40,287
Of those records, Hemoglobin result available within±7 days	27,150	33,077
Of those, patients with hemoglobin levels above 100 g/L	25,911	31,411
Of those, patients having hsCRP, total, LDL and HDL cholesterol, triglyceride and uric acid measurements one month within HbA1c level	12,594	14,020
Of those, patients having hsCRP ≤10 mg/L levels 9599 10,817 Of those, patients with HbA1c levels ≤5.6%	3699	4476

Table (3.2): Number of patients in database and those suitable for the analysis per age group

Patients' ages (years)	**Patients' number in database (men/women)**	**Patients suitable for analysis (men/women)**
20-<30	19968/50902	514/798
30-<40	27600/71040	1138/1357
40-<50	28310/39863	1467/1310
50-<60	34947/39419	2113/2096
60-<70	42961/44562	2395/2437
70-<80	28920/34159	1405/1851
80-<90	11350/18099	428/779
≥90	1208/2668	33/61

4. Results

4.1 Results of goal No. 1:

4.1.1 The effect of examined variables on HbA1c levels as measured by computed coefficient estimates

The impact of tested factors on HbA1c levels in terms of calculated coefficient estimates is presented on the (Fig. 4.1). Of note, in our population triglyceride levels exhibited the strongest association with HbA1c levels. HbA1c levels also increased with age and, at least in women, with total cholesterol levels. On the other hand, HDL cholesterol in women and uric acid levels in both genders seemingly associated with lower HbA1c values.

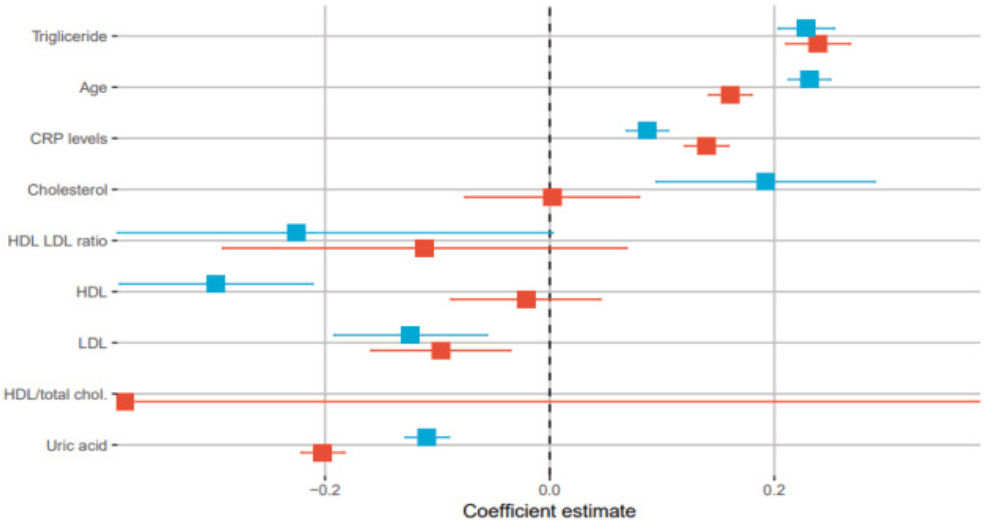

Figure (4.1): Calculated coefficient estimates when impact of tested factors on HbA1c levels was investigated. Red: male; blue: female patients. Horizontal lines indicate 95% confidence intervals [CI] (of note, in case of HDL/total cholesterol levels the numeric values of CI exceeded the represented area in both gender).

4.1.2 Variance-inflation factors for the studied parameters

Variance inflation factor (VIF) values of cholesterol, HDL and LDL indicated a very large interdependence. Given the common trend to order these tests simultaneously and the clinical use of these parameters, however, we decided to utilize all these parameters in one formula against accepted recommendations (table 4.1). For the development of the formula, median values and Q1, Q3 quartiles were used throughout all age cohort in both genders (Fig. 4.2).

Table (4.1): Variance-inflation factors (VIF) values indicating the relative independence of analyzed parameters in both gender. Note: higher VIF value refers to larger dependence

	Males	**Females**
Age	1.056	1.163
C-reactive protein level	1.087	1.135
Total cholesterol level	18.223	33.78
HDL/LDL cholesterol	8.161	16.408
HDL-cholesterol level	12.778	24.703
LDL-cholesterol level	13.717	19.785
HDL/total cholesterol	22.981	50.47
Triglyceride level	2.344	2.059
uric acid level	1.062	1.266

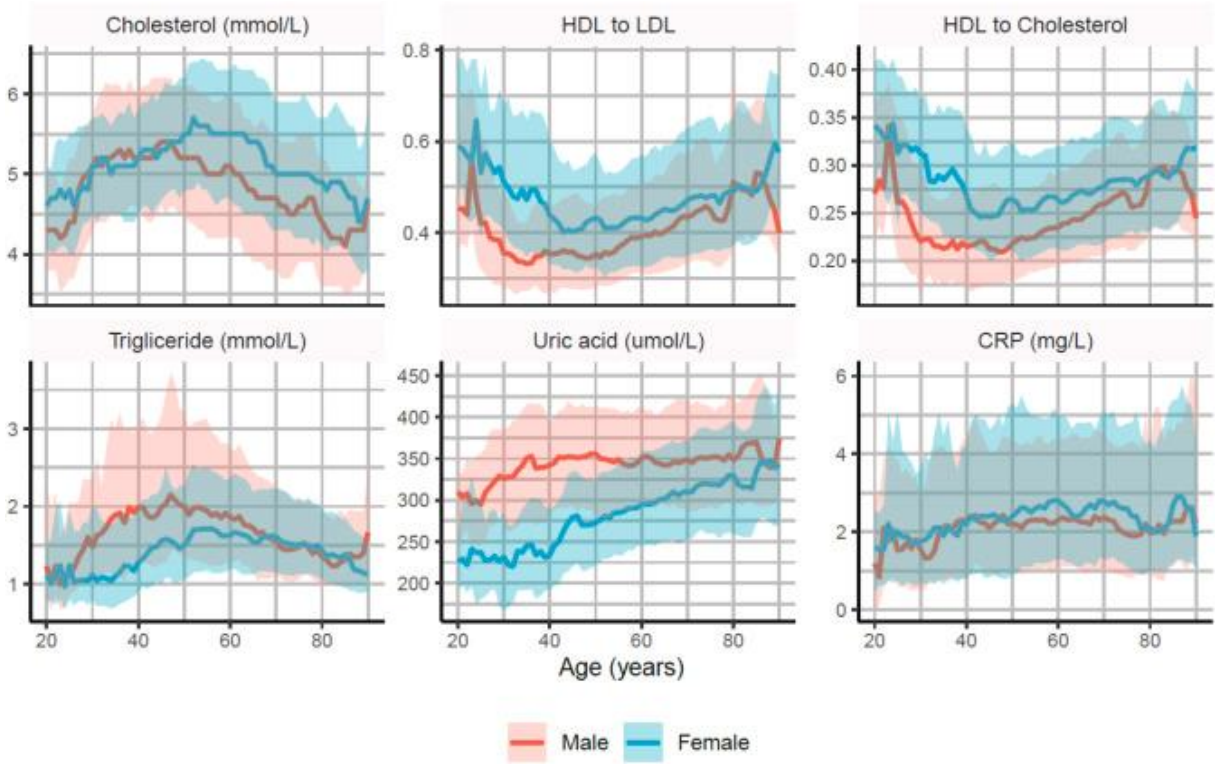

Figure (4.2): Median and quartile values of analyzed metabolites used for the development of formula throughout all age groups in females (red) and males (blue).

4.1.3 Correlation of HDL with HbA1c

In male patients, the correlation coefficient (R) between HbA1c% and HDL was– 0.16 which was statistically significant ($p<2.2e-16$); while in female patients, the

R value was – 0.24 which is also significant (p<2.2e-16). Thus a larger HDL levels tend to associate with lower HbA1c%, these findings are illustrated in the (Fig. 4.3).

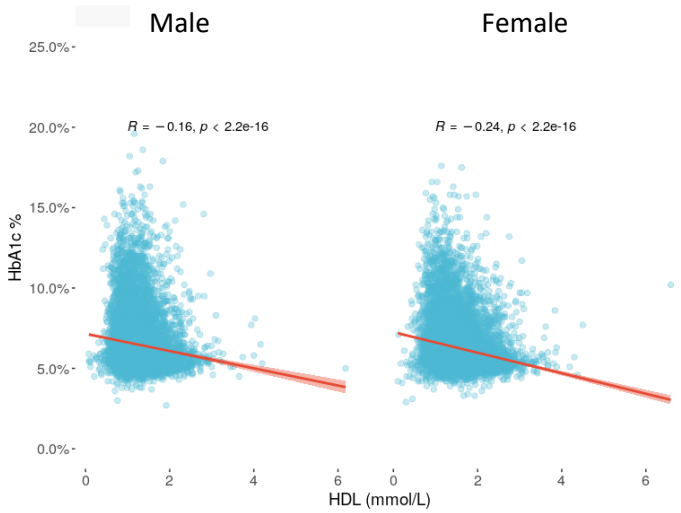

Figure (4.3): Correlation of HbA1c% versus serum HDL in males and females

4.1.4 Correlation of HDL/Cholesterol with HbA1c

The correlation coefficient (R) between HbA1c% and HDL/cholesterol in male and female individuals was – 0.091 and – 0.18, respectively. Both results were statistically significant (p 2.2e-16). These results are shown in the (Fig.4.4) which also revealed higher HDL/cholesterol levels which are generally associated with lower HbA1c%.

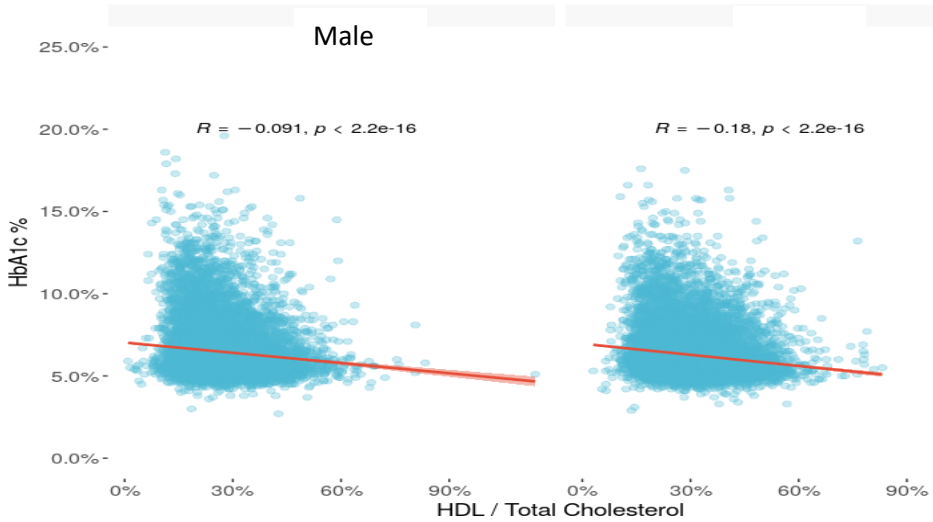

Figure (4.4): Males and females: HbA1c% vs serum HDL/Cholesterol correlation

4.1.5 Correlation of HbA1c with serum uric acid changes

The relationship between the degree of change in uric acid and the degree of change in HbA1c% level in patients have repeated HbA1c and their baseline HbA1c<7% revealed a correlation coeffienct (R) equal to 0.018 with no statistically significant differences (P=0.12) in relations between the changes in HbA1c% and the corresponding changes in serum uric acid as mentioned in the (Fig. 4.5).

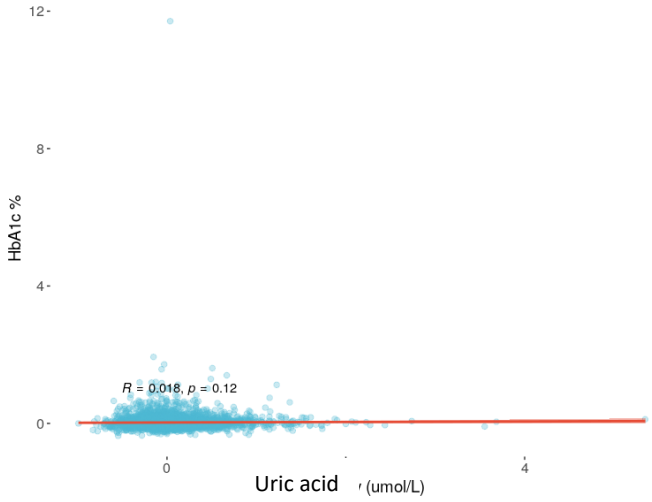

Figure (4.5): The levels of HbA1c% correlated to changes in serum uric acid levels

4.1.6 The impact of different variables on HbA1c% level

The (Table 4.2) describes separate multiple linear regression models for each gender to assess the impact of age, CRP, cholesterol, triglyceride and uric acid levels on HbA1c %. Although a unified model better captures all interactions, the presence of both categorical and continuous variables makes it harder to interpret. Values are not log-transformed in these models. Variance-inflation factors for male gender were: age: 1.054, CRP level: 1.06, CH level: 1.317, TG level: 1.272, URIC level: 1.027; while variance-inflation factors for female were: age: 1.141, CRP level: 1.09, CH level: 1.22, TG level: 1.194, and for Uric acid level: 1.16.

4.1.7 Correlation of changes in HbA1c % vs changes in serum uric acid levels

Pearson's correlation coefficients between changes in HbA1c levels and changes in uric acid levels among patients showed that R value (– 0 .1) indicates a weak correlation between the parameters but the association is statistically significant ($p<2.2e^{-16}$); comparable results were also estimated for log2 FC for HbA1c and log2FC serum

uric acid (R = – 0 .11, $p<2.2e^{-16}$). The number of observations might still make it possible to further explore the relationship via linear regression and gender distribution.

4.1.8 The correlation between HbA1c % and C-reactive protein

CRP level in males was significantly associated with HbA1c (p=1.305e-34) and the correlation in females were also statistically significant (p=6.485e-23), these findings are mentioned in the (Table 4.2).

Table (4.2): The effect of various factors on HbA1c% level

Parameter	Estimate	Std_error	p_value	CI_lo	CI_hi	Gender
(Intercept)	6.29843	0.07484	0.000e+00	6.15175	6.44510	Men
Age	0.01032	0.00078	1.002e-39	0.00879	0.01185	Men
CRP_level	0.00332	0.00027	1.305e-34	0.00279	0.00385	Men
CH_level	-0.02006	0.00948	3.433e-02	-0.03864	-0.00148	Men
TG_level	0.16993	0.00570	4.851e-191	0.15876	0.18110	Men
URIC_level	-0.00182	0.00011	4.457e-59	-0.00204	-0.00160	Men
(Intercept)	5.64726	0.06093	0.000e+00	5.52784	5.76668	Women
Age	0.01382	0.00065	1.303e-99	0.01255	0.01509	Women
CRP_level	0.00273	0.00028	6.485e-23	0.00219	0.00327	Women
CH_level	-0.07860	0.00867	1.351e-19	-0.09559	-0.06160	Women
TG_level	0.24269	0.00722	1.648e-241	0.22853	0.25684	Women
URIC_level	-0.00038	0.00010	2.514e-04	-0.00059	-0.00018	Women

4.1.9 Estimation of HbA1c levels based on complex metabolic parameters

The formula for the estimation of HbA1c levels in women is based on 10817 observations and is as follows: HbA1c (estimated) = 0.752 + 0.237*log10 (HDL/cholesterol) + 0.156*log10 (cholesterol) + 0.077*log10 (triglyceride) + 0.025*log10 (CRP) +0.001*log10 (age) -0.026*log10 (HDL/LDL) -0.063*log10 (uric acid)-0.075*log10 (LDL)-0.199*log10 (HDL)

The formula for the estimation of HbA1c levels in men is based on 9599 observations and is as follows: 1.146 + 0.08*log10 (triglyceride) + 0.046*log10 (CRP) + 0.01*log10 (cholesterol) + 0.001*log10 (age) -0.014*log10 (HDL)-0.018*log10 (HDL/LDL)-0.025*log10 (HDL/cholesterol) - 0.068*log10 (LDL) - 0.159*log10 (uric acid). where triglyceride, cholesterol, LDL and HDL cholesterol were expressed as mmol/L; uric acid levels as µmol/L, CRP as mg/L and HbA1c as %; log10 indicates 10-based logarithmic value. The estimated HbA1c values were compared to median levels of measured HbA1c levels. Estimated and measured values matched perfectly in women between 20 and 70 years of age, while estimated values exceeded constantly by 0.3 – 0.5% the measured values in men between 20 and 70 years of age. The level of significance for the difference (p values) between measured and estimated HbA1c levels for each age group and both gender is also calculated.

4.2 Results of goal No. 2:

4.2.1 The correlation coefficients between HbA1c levels and RDW

The results of the current study indicated a weak correlation (R=0.0037) between the HbA1c% values vs the RDW values of RBCs for the patients including both genders, however the relation is statistically significant (p=0.0062). The number of observations might still make it possible to further explore the relationship via linear regression in each gender separately; the input data contained 66408 males (27596 distinct) and 73345 female records (33896 distinct).

4.2.2 The correlation coefficients between HbA1c levels and MCV

Pearson's correlation coefficients between HbA1c levels and MCV revealed a negative weak correlation (R= – 0 .1) though still significant correlation ($p<2.2e^{-16}$). The model was not split by gender (Fig. 4.6); however, similar findings were also obtained when the values were separated according to the gender, (Fig.4.7).

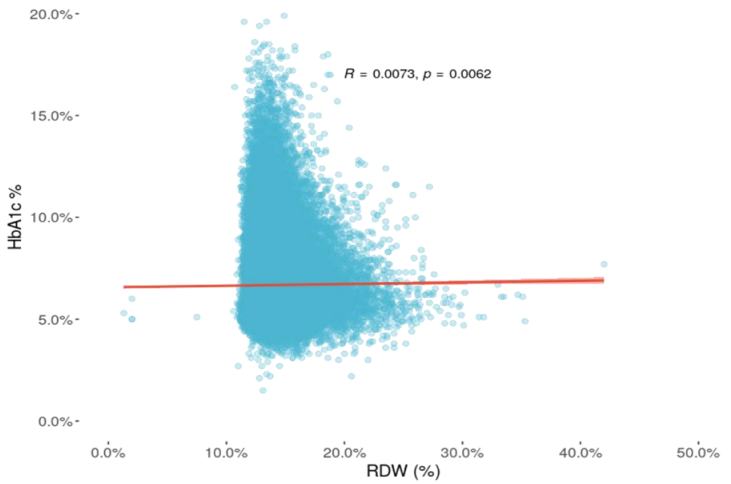

Figure (4.6): The association between RDW and HbA1c%

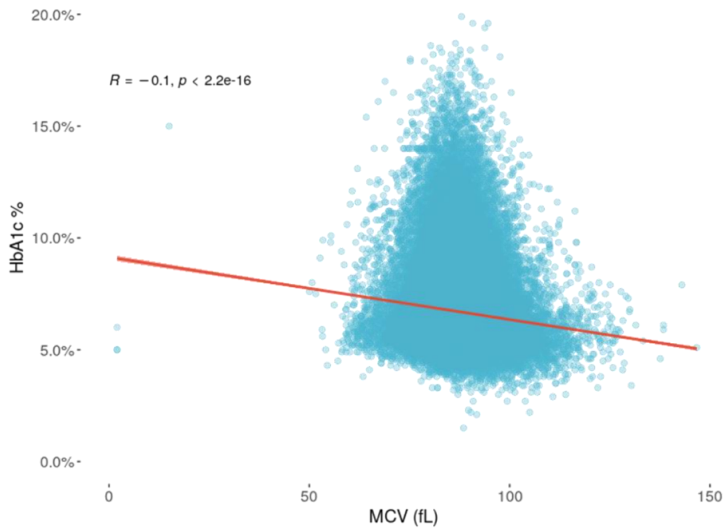

Figure (4.7): The association between MCV and HbA1c%

4.2.3 The correlation coefficients between HbA1c levels and hemoglobin

Hemoglobin levels and HbA1c levels showed a weakly negative but nonetheless statistically significant (p2.2e-16) association (Fig. 4.8), according to Pearson's correlation coefficients. The model was not gender-separated, but similar results were also obtained when the data were divided by gender.

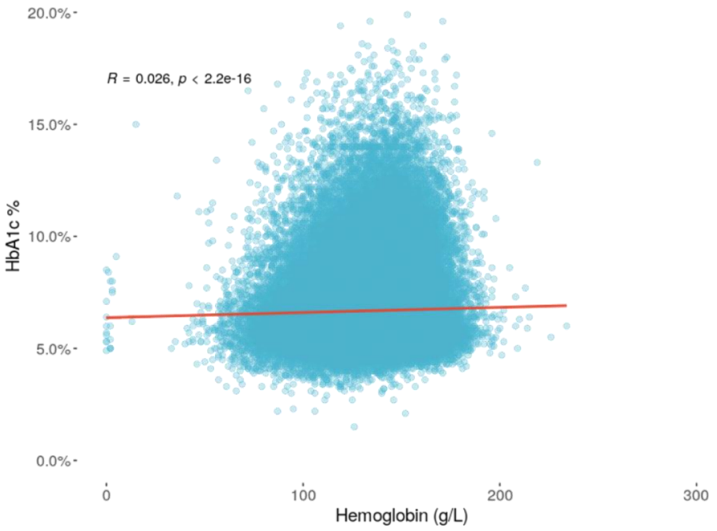

Figure (4.8): The relation between hemoglobin and HbA1c%

4.2.4 The Correlation of Hemoglobin <100 g/L and HbA1c%

Pearson's correlation coefficients between HbA1c% levels and hemoglobin under 100 g/L was measured and the model was not splitted by gender (Fig. 4.9). The association between the two parameters indicated a weak correlation (R value = 0.033) but statistically significant (p=0.018); the number of observations might still make it possible to further explore the relationship via linear regression.

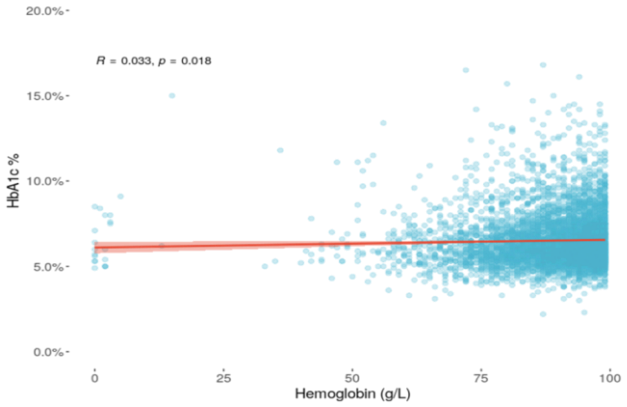

Figure (4.9): The association Between Hemoglobin Below 100 g/L and HbA1c%

4.3 Results of goal No. 3 (association of HbA1c with vitamin D)

Pearson's correlation coefficients between changes in HbA1c levels and changes in vitamin levels (percent change and log2 (Fold change)); the model is not split by

gender (Fig. 4.10). The R value (0 – 0.031) indicated a weak negative correlation between the parameters and the association was not statistically significant (p=0.12), and these findings were not changing [(R= 0 – 0.024), P=0.2] when log2FC vitamin D was replacing vitamin D; the number of observations might still make it possible to further explore the relationship via linear regression; the results revealed no significant association between changes in vitamin D and changes in HbA1c in males or in females.

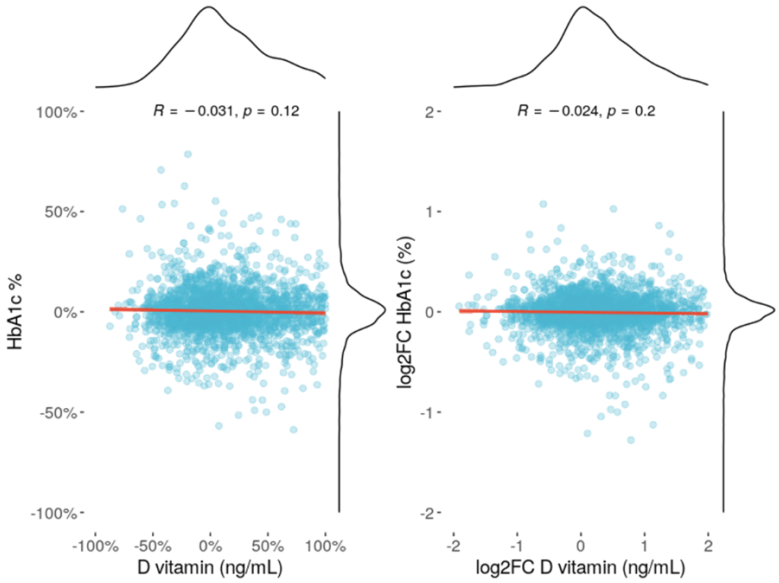

Figure (4.10): The association between changes in vitamin D and HbA1c%

The results of the current study showed a weak negative correlation (R= – 0.14) between HbA1c and serum vitamin D levels among study group and this correlation was statistically significant (p = 2.2-e16) as demonstrated in the (Fig.4.11); when the patients were sub-grouped according to their gender, the results revealed statistically significant association between HbA1c% and serum vitamin D in men and similar findings in females (Fig.4.12).

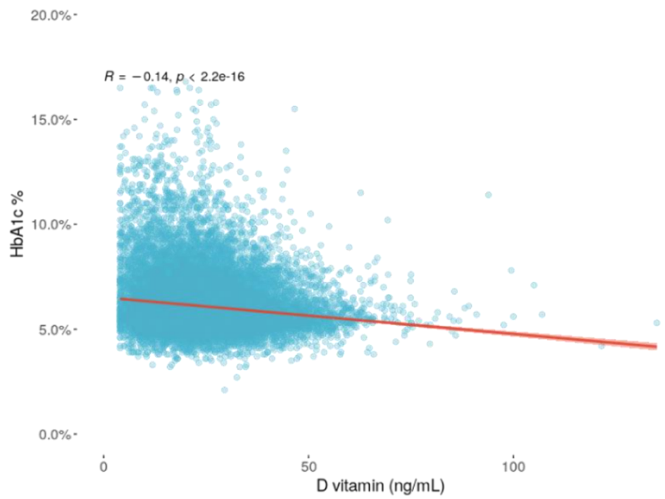

Figure (4.11): Association between concentrations of serum vitamin D and HbA1c%

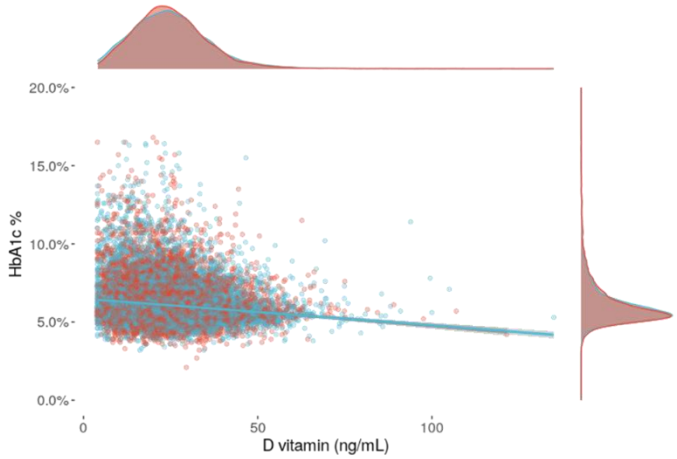

Figure (4.12): The association between concentrations of serum vitamin D and HbA1c% in males (red) and females (blue)

4.4 Results of goal No. 4 (association of HbA1c in different visits with age and gender)

The second HbA1c data group encompassed 193,165 individuals who received medical attention as outpatients and inpatients from 2006 to 2018. In terms of gender distribution, there were 92,700 (47.99%) males and 100,465 (52.01%) females, resulting in a ratio equal to 0.922:1.

4.4.1 Age distribution of the study population

The age range of the individuals was between 1-96 years, however, most of the study population in these HbA1c data were between 45 to 80 years old and mean 51.42 ± 22.36 years old. The HbA1c was done in descending manner for age group 61-80 years old, 41-60 years old, ≤20 years old, 20-24 years old, then ≥81 years old, in % equal to 36.6%, 28.1%, 16%, 14%, then 5.3 % respectively (Table 4.3); these differences in the %s of performing HbA1c among different age groups were statistically significant (P value=0.0001).

4.4.2 HbA1c results among the participants in the study

The mean HbA1c for all studied population was 6.8 ±1.699% with a range equal to 4.0-18.2%; 47.1% of the studied HbA1c values were in diabetic range (6.5% or higher), while the % of those in pre-diabetic (5.7% and 6.4%) or normal (<5.7%) were 26.8% and 26% respectively, this is shown in the (Table 4.4).

Table (4.3): Age groups of study population

Age group (years)	Frequency	% (%)
≤20	30888	16
21-40	26952	14
41-60	54357	28.1
61-80	70792	36.6
≥81	10187	5.3
Total	193176	100

Age group and HbA1c: (P value=0.0001)

Table (4.4): distribution of the studied population according to their HbA1c values

HbA1c category		
	Frequency	% (%)
Normal	50312	26
Pre-diabetic	51797	26.8
Diabetes	91067	47.1
Total	193176	100

4.4.3 The correlation between the HbA1c results and age groups

The results revealed that coefficient of determination (R2) between the HbA1c values and the patients' ages according to different age groups was positive weak

correlation (r^2=0.005); while the p value was statistically significant (p=0.0001). The highest %s of participants were having diabetic HbA1c category in four age groups, ≤ 20, 41-60, 61-80, and ≥ 81 years old; while the highest % individuals in the age group 21-40 years old were mostly in the normal category of HbA1c, these results are described in the (Table 4.5).

Table (4.5): The relationship between age groups and HbA1c results

Age group	HbA1c category					
	Normal		Pre-diabetic		Diabetes	
	Frequency	%	Frequency	%	Frequency	%
≤ 20	8546	27.67%	3723	12.05%	18619	60.28%
21-40	14009	51.98%	4743	17.60%	8200	30.42%
41-60	13816	25.42%	15982	29.40%	24559	45.18%
61-80	11958	16.89%	23618	33.36%	35216	49.75%
≥ 81	1983	19.47%	3731	36.63%	4473	43.91%

P value =0.0001

Similar findings were also reported when the patients' ages were correlated randomly with their HbA1c values, as the results showed a positive weak correlation between ages and HbA1c rate with (r^2= 0.055) with a highly significant relationship (P-value 0.0001), this is illustrated in the (Fig. 4.13).

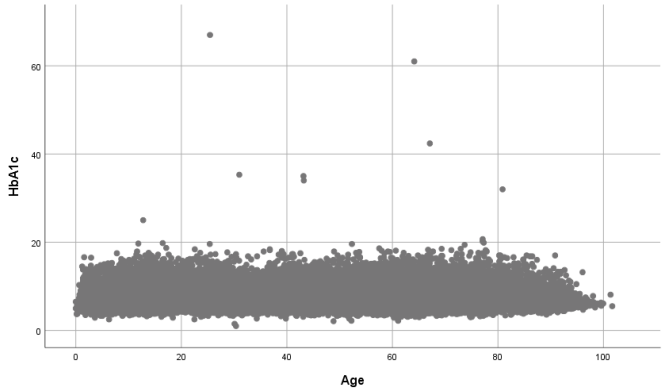

Figure (4.13): The correlation between HbA1c values and the patients' ages

4.4.4 Distribution of participants according to Sex and HbA1c

The gender distribution of HbA1c results revealed highest % of females (45.11%) were having diabetic category of HbA1c, while the females with normal or pre-diabetic categories were having lower %s, 27.84% and 27.05% respectively. The males were having nearly comparable results with 49.355 diabetic males, while 24.1%

normal males and 26.56% pre-diabetic males. These results were statistically highly significant (P-value = 0.0001); these findings are described in the (Table 4.6).

Table (4.6): The gender distribution of participants in regards to their HbA1c values

Gender	HbA1c category					
	Normal		Pre-diabetic		Diabetes	
	Frequency	%	Frequency	%	Frequency	%
Female	27971	27.84%	27174	27.05%	45320	45.11%
Male	22337	24.10%	24618	26.56%	45745	49.35%

P-value = 0.0001

4.4.5 Correlations of ages and gender with HbA1c values

There was a weak positive correlation between ages of males and HbA1c values (r^2=0.012); however, the relation was statistically highly significant (P-value 0.0001). For females, the correlation coefficient had weak positive relation with the HbA1c values (r^2=0.094), this association was statistically significant relationship (P-value = 0.0001); these results are shown in the (Table 4.7).

Table (4.7): The correlation between the ages of females and their HbA1c values

		HbA1c
Ages of males	Correlation Coefficient	0.012
	P value	0.0001
	Frequency	92700
Ages of females	Correlation Coefficient	0.094
	P value	0.0001
	Frequency	100465

When the study populations stratified into different age groups, the highest frequency, of those did two HbA1c tests at different interval, was in 61-80 [n=9,997 (36.6%)] while the least was in age group ≥81 years [n=1,213 (4.4%)], (Table 4.8).

Table (4.8): The distribution of participants according to different age groups

Age groups		
	Frequency	Percent (%)
Lower or equal to 20	3352	12.3
21-40	3890	14.3
41-60	8840	32.4
61-80	9997	36.6
More or equal to 81	1213	4.4
Total	27292	100.0

4.4.6 Categorization of HbA1c results according to patients' visits

The first HbA1c readings in study population was within normal range for 8,742 (32%), 6,483 (23%) individuals were having readings in prediabetes range, while 12,067 (44.2%) in the diabetic range. In the second HbA1c test for the same individuals, the normal range was recorded for 9,202 (33.7%), while prediabetes range was recorded for 6,536 (23.9%), while diabetes range was measured in 11,554 (42.3%), the (Table 4.9) and (Table 4.10).

Table (4.9): The frequency of individuals according to HbA1c results in the first visit

HbA1c groups 1		
	Frequency	Percent
Normal	8742	32.0
Pre-diabetic	6483	23.8
Diabetes	12067	44.2
Total	27292	100.0

Table (4.10): Frequency of individuals according to HbA1c results in the second visit

HbA1c groups 2		
	Frequency	Percent
Normal	9202	33.7
Pre-diabetic	6536	23.9
Diabetes	11554	42.3
Total	27292	100.0

The mean HbA1c reading in the first measurement was 6.9±2.02%, while for the second reading was 6.66±1.70 %. The range of HbA1c% in the first visit was 1.5 – 67%, whereas in the second visit became less (2.2 – 61%), as shown in the (Table 4.11).

Table (4.11): Statistics of the HbA1c results in the first and second visits

	1st visit HbA1c%	2nd visit HbA1c%
Mean	6.90	6.66
Std. Deviation	2.02	1.70
Range	65.5	58.8
Minimum	1.5	2.2
Maximum	67	61
Total number	27,292	27,292

Correlation between first visit readings with the second visit HbA1c readings was statistically significant with positive correlation, (P-value 0.0001), r^2 =0.765. this mean that there is aa positive relationship (r^2 =0.765) between the first visit HbA1c test result with the second HbA1c result with a highly significant relationship (P-value 0.0001).

The number of diabetic patients on the first visit who did not improve for the second visit has been divided into males and females for each age decade. The results revealed that highest % [n=1130, (24.0%)] of not improved females were in age group 60-69 years' old which is also was the age group that contained the highest % [n=340 (26.5%)] among all improved females in different age groups. Within each female age group, the best %s of improvement were observed in ages 70-79 (26.8%), ≥80 (26.0%), and 30-39 (25.5%) with improvement in HbA1c to levels less than diabetic levels. For males, the age group 60-69 years old included the highest % of non-improved (24.8%) and improved (29.0%) HbA1c readings in the second visit; while within each age group the best improvement was in age groups 70-79 (25.7%), ≥80 (25.4%), and 60-69 (24.8%), (Table 4.12).

Table (4.12): The second visit improvement in HbA1c according to different age groups among both genders from diabetic to pre-diabetic or normal HbA1c levels

				Improvement			
				No (not improved)		Yes (improved)	
				Count	%	Count	%
Sex	Female	Age group	Lower than 20	658	13.9%	64	5.0%
			20-29	190	4.0%	42	3.3%
			30-39	234	5.0%	80	6.2%
			40-49	396	8.4%	69	5.4%
			50-59	905	19.2%	251	19.6%
			60-69	1130	24.0%	340	26.5%
			70-79	873	18.5%	319	24.9%
			Equal or higher than 80	331	7.0%	116	9.1%
	Male	Age group	Lower than 20	698	14.7%	109	8.2%
			20-29	140	3.0%	14	1.0%
			30-39	277	5.9%	79	5.9%
			40-49	500	10.6%	140	10.5%
			50-59	1122	23.7%	323	24.2%
			60-69	1175	24.8%	387	29.0%
			70-79	634	13.4%	219	16.4%
			Equal or higher than 80	188	4.0%	64	4.8%

The number of all patients divided into males and females for each age decade according to their improvement in HbA1c values; the results showed that among all age groups, the females in age group 60-69 years old had the highest number of patients (n=2232) with HbA1c in diabetic range which also had the highest % of both not improved (20.8%) and improved (24.2%) HbA1c readings in the second visit; comparable values were also reported for the males; this is described in (Table 4.13).

Table (4.13): The improvement in HbA1c results in the second visit according to different age groups in each gender

				Improvement			
				No (not improved)		Yes (improved)	
				Count	%	Count	%
Sex	Female	Age group	Lower than 20	1378	12.8%	193	5.4%
			20-29	708	6.6%	125	3.5%
			30-39	1206	11.2%	269	7.5%
			40-49	1075	10.0%	344	9.7%
			50-59	1895	17.7%	696	19.5%
			60-69	2232	20.8%	861	24.2%
			70-79	1613	15.0%	762	21.4%
			Equal or higher than 80	624	5.8%	313	8.8%
	Male	Age group	Lower than 20	1447	15.0%	253	7.6%
			20-29	363	3.8%	67	2.0%
			30-39	775	8.0%	201	6.0%
			40-49	1136	11.8%	366	11.0%
			50-59	2156	22.3%	786	23.5%
			60-69	2182	22.6%	925	27.7%
			70-79	1220	12.6%	580	17.4%
			Equal or higher than 80	377	3.9%	164	4.9%

The mean HbA1c was highest (7.43±2.84%) in age group <20 years' old and still the age group of highest reading mean (6.79±2.08%) in the second visit. The age group 30-39 years old was the age group of lowest mean in HbA1c in the first visit (6.34±1.91%) and it recorded the lowest mean HbA1c in the second visit (6.19±1.6%), (Table 4.14).

Table (4.14): Effect of age subgroups on HbA1c result

		HbA1c 1st visit		**HbA1c 2nd visit**		**P-value**
		Mean	SD	Mean	SD	
Age groups	Lower than 20	7.43	2.84	6.79	2.08	0.0001
	20-29	6.54	2.78	6.32	1.89	0.0001
	30-39	6.34	1.91	6.19	1.60	0.160
	40-49	6.78	2.07	6.64	1.75	0.682
	50-59	6.96	1.85	6.77	1.59	0.0001
	60-69	6.94	1.73	6.74	1.70	0.0001
	70-79	6.88	1.61	6.70	1.44	0.0001
	Equal or higher than 80	6.83	1.54	6.70	1.50	0.0001

In the first visit the highest %s (57.4%) for normal HbA1c values were reported in age group 30-39, for pre-diabetic readings in age group ≥80 (31.7%), and for diabetic HbA1c values, in age group 70-79 (49.0%). In the second visit, the highest HbA1c readings were recorded in age group 20-29 years old (58.3%), (Table 4.15).

Table (4.15): Effect of age subgroups on HbA1c categories

		HbA1c 1st visit						HbA1c 2nd visit					
		Normal		Pre-diabetic		Diabetes		Normal		Pre-diabetic		Diabetes	
		Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Age groups	Lower than 20	1396	42.7	346	10.6	1529	46.7	1556	47.6	236	7.2	1479	45.2
	20-29	722	57.2	155	12.3	386	30.6	736	58.3	125	9.9	402	31.8
	30-39	1406	57.4	375	15.3	670	27.3	1420	57.9	383	15.6	648	26.4
	40-49	1176	40.3	640	21.9	1105	37.8	1177	40.3	630	21.6	1114	38.1
	50-59	1496	27.0	1436	26.0	2601	47.0	1480	26.7	1546	27.9	2507	45.3
	60-69	1398	22.5	1770	28.5	3032	48.9	1498	24.2	1860	30.0	2842	45.8
	70-79	838	20.1	1292	30.9	2045	49.0	967	23.2	1308	31.3	1900	45.5
	Equal or higher than 80	310	21.0	469	31.7	699	47.3	368	24.9	448	30.3	662	44.8

The (Table 4.16) compares the HbA1c results mean of two visits between males and females and highly significant differences (p value = 0.0001) for each male and female two visits observed also there was highly significant differences (p value = 0.0001) between sex variables on the HbA1c results mean of two visits.

Table (4.16): Effect of sex on HbcA1 results in the two visits

Sex	**HbA1c 1st visit**		**HbA1c 2nd visit**		**P-value**	**P-value**
	Mean	SD	Mean	SD		
Female	6.80	1.99	6.60	1.67	0.0001	0.0001
Male	7.01	2.04	6.73	1.72	0.0001	

Males with pre-diabetic HbA1c values increased in the second visit (n = 3092) compared to the first visit (n = 2968); similar outcomes were found for males and females in the normal HbA1c range, while the number of patients in the diabetic range decreased for both genders, as shown in the (Table 3.17).

Table (4.17): The effect of sex on HbcA1 categories among two visits

		HbA1c 1st visit						HbA1c 2nd visit					
		Normal		Pre-diabetic		Diabetes		Normal		Pre-diabetic		Diabetes	
		Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Sex	Female	4781	33.4	3515	24.6	5998	42	5125	35.9	3444	24.1	5725	40.1
	Male	3961	30.5	2968	22.8	6069	46.7	4077	31.4	3092	23.8	5829	44.8

The %s of men individuals with change in category in HbA1c% according to their age groups revealed that age groups 20-29 years and 70-79 years had lowered HbA1c% in the second. all the other age groups had increased in %s of patients with increased HbA1c in the second visit when compared to the first one, this is shown in the (Table 4.18). While for women, the distribution of patients according to their changes in the second visit readings of their HbA1c% results revealed that in age groups 20-29 years, 30-39 years, 70-79 years, and age group ≥ 80 years had higher %s of individuals, this is described in the (Table 4.19).

Table (4.18): The frequency of men patients of HbA1c % in their second visit

Age (men)	First visit: HbA1c >7% (100%)		Frequency of Lowered HbA1c level in the second visit		Frequency of increased HbA1c level in the second visit	
	Frequency	%	Frequency	%	Frequency	%
20-29	989	11.86	965	14.76	735	11.38
30-39	292	3.50	203	3.11	227	3.51
40-49	681	8.17	467	7.14	509	7.88
50-59	975	11.69	710	10.86	792	12.26
60-69	1861	22.31	1425	21.80	1517	23.48
70-79	1962	23.53	1600	24.48	1507	23.32
Equal or higher than 80	1208	14.48	883	13.51	917	14.19

Table (4.19): The frequency of women patients of HbA1c % in their second visit

age (women)	First visit: HbA1c >7% (100%)		Frequency of lowered HbA1c in second visit		Frequency of increased HbA1c in second visit	
	Frequency	%	Frequency	%	Frequency	%
20-29	918	9.40	855	12.11	716	9.90
30-39	650	6.65	423	5.99	410	5.67
40-49	1216	12.45	634	8.98	841	11.63
50-59	1048	10.73	608	8.61	811	11.21
60-69	1737	17.78	1252	17.73	1339	18.51
70-79	2040	20.88	1548	21.93	1545	21.36
Equal or higher than 80	1538	15.74	1249	17.69	1126	15.57

5. Discussion

5.1 Goal No. 1: The relationship between HbA1c levels lipid profile, uric acid, and C-reactive protein

There was significant negative correlation between HbA1c% and serum levels of HDL-C, while TC, TG, and LDL-C were having significant positive correlation with HbA1c%. One of the limitations in this study was the exact diseases of the hospitalized patients were not specified; however, since some of them have HbA1c% higher than 7, it seems that some of the patients were diabetics. Paradoxically, this limitation may notify the presence of inpatients with normal HbA1c% and elevated HbA1c% but still the correlation in significant in all of them, which clarify the presence of significant association between HbA1c% and lipid parameters in both, non-diabetic people and diabetics.

Insulin favour lipid conservation in the tissues by decreasing fatty acid release from adipose tissues and accelerate TG synthesis in the tissue and shift TG from blood to the adipose tissue (Dimitriadis G, 2011); Thus, an increase in HbA1c%, which indicates an anomaly in insulin synthesis or function, will be linked to an increase in TG, LDL, and TC.

The current study found a negative correlation between the HbA1c% and HDL. Huang R. et al. (Huang R, 2021), also found a significant negative correlation between HDL-C and glycosylated hemoglobin in their cross-sectional study of diabetic patients. These findings suggest that lower HDL-C may be associated with poorer glucose control in diabetic patients and may increase the risk of cardiovascular diseases. Numerous lipids and proteins found in HDL-C have the capacity to protect vessels and have anti-diabetic effects on cells. Adults with low HDL-C, particularly those with diabetes, have been linked to an increased risk of coronary heart disease and stroke, according to pertinent literature. There is a negative correlation between cardiovascular illnesses and HDL-C. Therefore, it is advised that individuals with low HDL and elevated HbA1c% get evaluated for the potential of vascular disorders, particularly CVA and IHD. Beyza C. et al. (Beyza Çiçek, 2023), hypothesized that HbA1c can be utilized to assess cardiovascular risk in non-diabetic patients even in the early stages of coronary artery disease.

The results of Begum A, et al (Begum A, 2019) were comparable to our findings for TC, TG and HDL-C but not for LDL-C in diabetic patients. The results of Shanmuga Priya and her colleague were in line with our results (Shanmuga Priya, 2020). Al shaheeb, et al, assessed lipid profile with HbA1c in type 2 diabetic Iraqi patients and reported results in consistence with our findings (Al shaheeb, 2022).

Ahmed Mahdi Habeeb et al. discovered, in contrast to our findings, that diabetes mellitus has no discernible effects on cholesterol, HDL, triglycerides, or LDL; rather, only those with greater VLDL were more likely to have higher HbA1c% in patients with DM than those in the control group (Ahmed Mahdi Habeeb, 2023).

Abdulwahed H. M. revealed that lipid profile of the diabetic patients with hemoglobin A1c <7.0 and those with hemoglobin A1c > 7.0 differed significantly; >7.0 had considerably higher levels of LDL, triglycerides, and cholesterol. He alerts us to the presence of association between HbA1c and lipid profile only when HbA1c is higher than what is considered to be normal (Muhammed., 2023). Alzahrani SH, et al. reported significant association of HbA1c% only with TG and not with other lipid parameters, (Alzahrani SH, 2019).

According to the study's findings, there was positive correlation association between CRP level and HbA1c in men patients was statistically significant, and it was also present in women. However, the inflation factor for CRP was1.085 which indicated low association and very little multicollinearity with HbA1c. The patients were randomly selected from electronic hospital data; they were complaining from different diseases; it seems that many of them have DM as HbA1c above 6.5 % was not uncommon (Bahceci, 2005), reported positive correlation between HbA1c and CRP in diabetic patients and in non-diabetics; (Gautam, 2023), and (Khadije Ahmad, 2021), recorded higher HbA1c levels are associated with increased CRP in poorly controlled diabetes mellitus. Positive correlation between HbA1c and CRP in DM is also reported in many other studies (Seo YH, 2021), (Meriga RK, 2020), (Shi, 2015), (Gupta, 2019), (Shen Q, 2023), (Vinod A, 2023), (Sarinnapakorn V, 2013), (Ajay Meshram, 2013), Jeong Woo Seo and his colleague found that HbA1c is better correlated with CRP than fasting blood sugar (Jeong Woo Seo, 2021). The increase in CRP among DM patients with high HbA1c may be a possible risk factor for acquiring other illnesses like cardiovascular diseases. The positive correlation between HbA1c and CRP is through a

number of mechanisms, as chronic hyperglycemia is thought to contribute to inflammation by activation of inflammatory pathways. In turn, inflammatory activities may decrease glycemic control and lead to insulin resistance (Seo YH, 2021).

In this study, patients with repeated HbA1c measurement and have a baseline HbA1c<7%, the association between the degree of change in uric acid and the degree of change in HbA1c% level was found to be minimal, with no statistically significant differences. However, if the % of HbA1c is higher than 7%, there is a small but significant negative association between HbA1c and uric acid; this finding holds true for both genders. A growing body of research suggests that the quantities of uric acid and glucose fit like a "bell." Following their initial rise, uric acid levels typically decline as blood glucose concentration rises. Numerous investigations have discovered an inverse relationship between type 2 diabetes patients' blood glucose concentrations and serum uric acid levels (D. G. Cook, 1986), (T. P. Whitehead, 1992), (Ford., 2008). But up until now, it hasn't been apparent why this association occurs or what circumstances affect it.

According to Yuliang Cui et al., there is an inverse relationship between uric acid and HbA1c in individuals with recently diagnosed type 2 DM These results are consistent with our findings; however, they discovered that serum uric acid was negatively linked with HbA1c only in the event of high insulin levels. In individuals with low insulin levels, there was no correlation between uric acid and HbA1c. However, regardless of blood glucose level, there are no variations in serum uric acid levels if patients had normal insulin levels; therefore, insulin levels are probably a determining factor in the association between uric acid and HbA1c (Cui Y, 2016). Rusdiana, et al. (Rusdiana, 2018) discovered no evidence of a significant correlation between uric acid levels and HbA1c in DM patients.

Wei, F., et al found that in T2DM patients, serum uric acid level has an inverse relationship with HbA1c; while in persons with normal blood sugar, there is a positive correlation. They suggested that reverse transfer of uric acid and glucose in renal tubules appears to be responsible for the correlations between serum uric acid and HbA1c (Wei, 2016). Results collected by Alaa A. and her colleague Ahmed K. indicated that serum uric acid is much greater in diabetic patients than in the general population; and that there is a significant positive association between HbA1c and

serum uric acid for patients with diabetes (Alaa A. F., 2019) which is comparable to our results. Moreover, in line with the current study results, Muhammad F. discovered a substantial correlation between the level of serum uric acid and HbA1c, fasting, and postprandial blood glucose, indicating a role for serum uric acid in the reduction of glucose tolerance (Muhammad F. H., 2014).

Surprisingly, the estimated HbA1c values exhibited a complete numeric match with the measured median HbA1c levels between 20 and 70 years of age in female patients. This finding indicates in this age group that any of the tested analytes can be estimated by taking the other analytes into account. Therefore, the question is challenging that the information provided by HbA1c levels can be regarded to be independent at least at population level. (Assessing our results one should note that individual variation may still allow large discrepancies between measured analytes.) In men of the same age range measured median HbA1c levels (while still correlated strongly with estimated HbA1c results) were systematically 0.5% below the estimated HbA1c levels. This indicates that some unidentified factor additionally to lipid profile, uric acid and CRP may have a systemic effect on the complex interplay between these parameters. Hence, in men HbA1c levels probably has some further determinant additionally to that of common metabolic parameters.

Based on the limited data of analytes included in our analysis the basis of this gender-dependent difference is still to be elucidated. (One should remember, however, that the inverse association between HDL and HbA1c levels was not detected in men, contrary to women.) Of note, the performance of our developed formulas deteriorated significantly in female patients over 70 years of age either, as estimated and measured HbA1c values diverged markedly. This supports the notion that in older female populations the information provided by HbA1c levels is rather independent from metabolic parameters. Due to the retrospective nature of our analysis our results do not allow to decide whether the correlation is causative (i.e. by modifying lipid metabolic profile or uric acid levels one can alter HbA1c levels) or casual (i.e. each parameter depends on a still unclarified parameter). Literary data, however, support that the modification of lipid or uric acid metabolism or inflammation may have an impact on glucose homeostasis characterized by HbA1c levels. (Kosmas CE, 2018), (Chen J,

2000), (Li D, 2023). It is important to note that the formulas developed are specific for the population analyzed.

A limitation of our study that we had no information about patients' diagnoses including the disturbed glucose homeostasis; therefore, we were not able to investigate whether the association between metabolic parameters and age alters in different subgroups of patients. As the evaluated population was probably heterogeneous in term of the presence of diabetes, we aimed to avoid the bias presented by the over-representation of diabetic patients (who are commonly referred to HbA1c measurements). Therefore, we enrolled each patient just once into our analysis and just the first record of their university laboratory history was used. As the majority of subjects exhibited elevated HbA1c levels (the 5.6% cut-off value for HbA1c levels in the diagnosis of diabetes, recommended by the American Diabetes Association was exceeded in about 60% of patients) (Strandberg TE, 2000), diabetes was probably overrepresented in the investigated population. It is also worth of mention, that in the analyzed period between 2008 and 2018 HbA1c was dominantly ordered for monitoring of diabetes. In addition, in order to avoid the interference with acute severe inflammation and severe anemia, patients with highly elevated CRP levels and very low hemoglobin levels, respectively, were also excluded One should also emphasize that in our analysis HbA1c levels and clinical chemistry parameters were expressed as per cent values instead of mmol/mol units and as mmol/L instead of conventional units, respectively. If laboratory parameters are expressed in other units, numeric values of factors in the equation will change; however, the phenomenon of strong relationship between routine metabolic parameters and HbA1c value will be the same.

5.2 Goal No. 2: The association between HbA1c levels and blood indices

In the current study, hemoglobin levels and HbA1c levels exhibited a weakly negative but statistically significant connection; the model was not gender-separated; however identical results were found when the data were divided by gender. In line with our results, Lai, Y., et al. (Lai, 2021) discovered that Hb levels were independently and negatively related to HbA1c levels in both men and women, yet they examined normal adults.

In accordance to our results, Bae JC, et al (Bae JC, 2014) found that in both men and women, the HbA1c value at a particular fasting plasma glucose level varied

according to hemoglobin level; they found that at the same fasting glucose level, participants with lower hemoglobin had greater HbA1c, and this finding was constant across the nondiabetic range of fasting plasma glucose. The HbA1c level increased by about 0.1%, correlating with a 2 g/dL decrease in hemoglobin at the same fasting glucose level in patients who did not have anemia. HbA1c% levels had a weak positive link but were statistically significant with hemoglobin levels less than 100 g/L; the results were likewise statistically significant among males and females. Thus a decrease in the total amount of hemoglobin in the blood can affect the HbA1c measurement. This is because the amount of HbA1c is proportional to the amount of hemoglobin available for glycation. If hemoglobin levels decrease, there is less hemoglobin available for glucose to bind to, which can result in a higher % of glycated hemoglobin, leading to an elevated HbA1c. Conversely, conditions that increase the lifespan of red blood cells, such as certain forms of anemia, can result in a falsely lower HbA1c, as the red blood cells have more time to accumulate glucose.

In this study, the HbA1c levels had significantly negative association with MCV and RDW. Other studies showed diverse results; Hardikar et al. (Hardikar P, 2012) observed an inverse relationship between HbA1c and MCV (r = -0.22, p 0.05), MCH (r = -0.30, p 0.05), and MCHC (r = -0.32, p 0.05) in non-diabetic participants. While Koga et al. discovered that HbA1c was negatively linked with MCV (r = -0.368, p 0.0001) and MCH (r = -0.320, p 0.0001) in premenopausal women, postmenopausal women showed no such relationship between HbA1c and MCV (r = -0.019, p = 0.771) and MCH (r = -0.104, p = 0.107) (Koga M, 2007). Bhutto AR, et al, found that RDW has a substantial association with HbA1c and is a cheap and easily accessible test, therefore it might be utilized as a glycemic status marker (Bhutto AR, 2019).

5.3 Goal No. 3: The correlation between HbA1c and serum vitamin D levels

The current study's results also revealed a weak, though significant, negative correlation between HbA1c and serum vitamin D levels among the study group, and when the patients were divided into subgroups based on their gender, the results showed a statistically significant association between HbA1c% and serum vitamin D in men and similar findings in women. Vitamin D deficiency is considerably common among otherwise healthy Iraqi people, primarily because of sedentary lifestyles, low dietary vitamin D supplementation, low levels of education, and insufficient sun exposure. The

common vitamin D deficiency and the elevated HbA1c in the study populations as some of them were diabetics may be in part responsible for the negative correlation in the present study.

Vitamin D is thought to improve glycemic control because it can reduce peripheral insulin resistance, alter inflammatory responses and immune responses, and increase the production of insulin (Park S, 2016). The results of Alqahtani R. and her colleague were comparable to ours (Alqahtani R, 2023). Moreover, to a meta-analysis by Farahmand, M.A., et al., comprising 2149 control subjects and 2164 intervention participants, vitamin D supplementation may help patients with type 2 diabetes who have low vitamin D status reduce their FPG, HbA1c, and HOMA-IR; these results show a strong correlation between low vitamin D and elevated HbA1c (Farahmand MA, 2023).

Several studies have found a link between vitamin D levels and glycemic management, although the link is not constant. Several animal and human research (Mitri J, 2014), (Pittas AG, 2010) suggest that vitamin D may be a risk modulator for both forms of diabetes. Some investigations indicate that vitamin D has a direct effect on insulin secretion and action, and hence on glucose homeostasis, by activating the vitamin D receptor on the -cell of the pancreatic islet of Langerhans (Pittas AG, 2007). Other research suggests that vitamin D plays an indirect role in the pathogenesis of both forms of diabetes by regulating calcium homeostasis and thereby influencing multiple mechanisms (such as pancreatic beta-cell dysfunction, poor insulin action, and systemic inflammation) (Al-Shoumer KA, 2015).

5.4 Goal No. 4: Exploring the fluctuation of glycated hemoglobin A1c levels in people during visit-to-visit

The study observed patients aged 1 – 96 years old, showing a wide age range. However, the majority of patients were between 45 and 80 years old, indicating that adults in this age group are most commonly screened for or affected by hyperglycemia, with a mean age of 51.42 ± 22.36 years. Aging, along with genetic and lifestyle-related risk factors, plays a role in the development of type 2 diabetes by affecting B-cell activity and their ability to adjust to insulin resistance, ultimately resulting in reduced insulin production (Galicia-Garcia U, 2020), (Lee PG, 2017). Research in rodents and

people has shown that aging can have a unique impact on b-cell regeneration and performance (JA., 2013).

The descending order of HbA1c testing across different age groups shows the highest % in the 61-80 age group (36.6%) and the lowest in the ≥81 years' age group (5.3%). These results are consistent with the assumption that older patients are more likely to have hyperglycemia and require more testing. However, in individuals over 80 years old, HbA1c testing decreases, which might reflect the restricted life expectancy and possible advantages of HbA1c testing in the really elderly; and thus clinicians may prioritize interventions with more immediate impact on health for individuals with limited life expectancy and other significant health conditions. Furthermore, clinicians may prioritize addressing other health concerns that have a more immediate effect on the patient's overall well-being and quality of life. Additionally, physicians may reduce their use of HbA1c testing in older patients because the diagnostic accuracy of HbA1c for diabetes decreases with age, primarily due to the decline in red blood cell count. Therefore, HbA1c is not ideal for diagnosing diabetes in elderly individuals due to their naturally lower RBC count (Wu L, 2017).

In this study, the study population had an average HbA1c of 6.8 ±1.699%. This suggests that a significant number of patients may have diabetes and require immediate management for their high blood sugar levels. Upon analyzing the HbA1c results of over 92,000 patients, it was found that 47% of them had HbA1c levels in the diabetic range, indicating poor blood sugar control and highlighting a high prevalence of diabetes among individuals seeking medical care at the hospital. The 47% of those in diabetic range is high when compared to previous studies; a previous Hungarian study reported a 26.68% moderate and high DM risk after measuring the HbA1c in a sample of 551 subjects (Galvács H, 2021), in Singaporean study, the prevalence of diabetes mellitus in a hospital-based study population is 19% (Teo LM, 2020), which is higher than the total adult population of Singapore at 14.2%. In Yong et al.'s study, participants aged 55 and older had a diabetes mellitus prevalence of 30% (Yong PH, 2018).

Approximately one quarter of the participants exhibited borderline HbA1c levels, putting them at risk of developing diabetes mellitus. They require prompt medical care to prevent the onset of diabetes mellitus. Diabetes mellitus is a relentless disease with long-term consequences if not well treated. A high initial HbA1c level in

the diabetic range is linked to the advancement of diabetes within a 3-year period. Disease progression was characterized by transitioning to sustained insulin therapy or having a HbA1c level exceeding 8.5% while being treated with two or more oral hypoglycemic agents. Patients with prediabetes have a 5–10% annualized conversion rate to diabetes mellitus, with comorbidities such as nephropathy and retinopathy starting to emerge at this stage (Tabák AG, 2012). On the other hand, those with HbA1c levels below 6% had a decreased risk of long-term problems (IM., 2000).

When examining the distribution of HbA1c categories by age, it is noted that the greatest proportions of individuals with diabetic HbA1c levels are present in the age groups < 20 (60.28%), 41-60 (45.18%), 61-80 (49.75%), and > 81 years (43.91%). Diabetes prevalence appears to be elevated in these particular age groups. The age group 21-40 years old has the highest % (51.98%) of individuals with normal HbA1c readings, suggesting a decreased prevalence of diabetes in this age range. The data indicate a significant occurrence of diabetes mellitus in children and teenage Hungarian patients, with the age of onset being identified as an independent risk factor for diabetes mellitus.

The weak correlation indicates a statistically meaningful link between age and HbA1c readings. However, the limited correlation suggests that age may not be a reliable predictor of HbA1c values, and other factors may be influencing the heterogeneity in HbA1c levels within each age group. Comparable to our study, prior studies have demonstrated a favorable correlation between HbA1c levels and age in several ethnic groupings (Arnetz BB, 1982), (Hashimoto Y, 1995), (Yang YC, 1997), (Carrera T, 1998), (FQ., 1999).

45.11% of females are categorized as diabetic based on their HbA1c levels. Among males, 49.355% fall into the diabetes category. Female individuals have a lower representation in the normal (27.84%) and pre-diabetic (27.05%) groups, similarly, men exhibited a comparable trend, with 24.1% falling into the normal category and 26.56% in the pre-diabetic category.

The results revealed that in each gender, there was a strong statistical relationship between the frequency of patients and their HbA1c categories. The results are statistically highly significant with a p-value of 0.0001. This indicates that the distribution of HbA1c categories among each gender is unlikely to be random, and

additional risk factors are contributing to the worsening glycemic control. Previous researches indicated that HbA1c values were notably higher in males compared to females across all subjects (Huang S.H., 2021), (Lee H.S, 2019), (Ma Q, 2016). The gender can indirectly influence the HbA1c by affecting the BMI. Women have lower fasting plasma glucose levels and higher 2-hour plasma glucose levels compared to men following an oral glucose tolerance test. Sex and gender play a crucial role in medicine, with distinct variances observed in glucose regulation, pre-diabetic conditions, as well as type 1 and type 2 diabetes. Sex hormones partially contribute to these sex disparities (F., 2018).

The study found a little positive correlation between the ages of males and HbA1c values, with a correlation coefficient (r^2) of 0.012. Similarly, females show a weak positive correlation with a r2 value of 0.094. Both situations have a weak correlation, indicating that the relationship between age and HbA1c levels is not strong. The statistical significance is clear because of the very low p-values for both groups (P-value = 0.0001), despite the weak correlation. A low p-value indicates that the observed association is unlikely to be a result of random variation. Roth J, et al. and Gülsen Ş, et al. also reported similar findings (Roth J, 2016), (Gülsen Ş, 2023).

Individuals in the age group of 60-69 years may exhibit improved response to treatment as a result of characteristics such as higher compliance with medication, more consistent lifestyle habits, and maybe fewer additional medical conditions affecting the effectiveness of treatment. On the other hand, individuals in the younger age group (20-29 years) may exhibit distinct lifestyle patterns, difficulties in following treatment plans, or physiological issues that can impact their reaction to treatment. Gender disparities in the improvement of HbA1c levels may be attributed to differences in hormonal effects, healthcare-seeking behaviors, or responsiveness to therapy between males and females. For example, variations in hormone levels in females may have a distinct effect on glucose metabolism and responsiveness to treatment compared to males. In addition, socioeconomic considerations, healthcare accessibility, and social support systems may also play a role in the gender differences observed in treatment outcomes.

The impact of treatment options and rates of adherence among various age and gender groups may also be influential in elucidating the observed disparities in HbA1c improvement. Demographic groupings may differ in factors such as medication

adherence, dietary compliance, physical activity levels, and access to healthcare facilities, which might impact treatment outcomes.

Differences in the initial severity of the disease, the length of time a person has had diabetes, and the existence of other health conditions among various age and gender groups may lead to varying reactions to therapy. Older adults may have more consistent illness trajectories or experience fewer problems, rendering them more receptive to therapy in contrast to younger folks who may face a more aggressive disease progression or greater health obstacles.

Examine the possible connections between age, gender, and the specific therapy methods or interventions used in this study. Specific demographic groups may exhibit varied responses in HbA1c improvement due to the varying effectiveness or tolerance of certain medicines or lifestyle modifications.

The absence of substantial progress in the 40-49 age bracket may be attributed to the length of time a person has had diabetes and their compliance with treatment. Individuals within this specific age group may have been diagnosed with diabetes for a prolonged period, resulting in more deeply rooted metabolic abnormalities that are difficult to manage using conventional treatments. In addition, medication adherence may differ among different age groups, with younger patients potentially encountering more difficulties in following treatment regimens.

The variation in treatment response among different age cohorts underscores the significance of tailored drugs in the management of diabetes. Customizing treatment approaches based on unique patient characteristics, such as age, can enhance the efficacy of interventions and maximize results.

Qu F, et al. discovered that persons with type 2 diabetes who had the highest levels of HbA1c variability were associated with increased risks of all-cause mortality, cardiovascular events, progression to chronic renal disorders, amputation, and peripheral neuropathy. Individuals diagnosed with type 2 diabetes who have a high degree of variability in their HbAlc measurements are at a greater risk of developing kidney failure, progressing to albuminuria, experiencing diabetic foot ulcers, and developing retinopathy. The risks of all events, with the exception of amputation and retinopathy, were found to rise with each incremental standard deviation of HbAlc variability measures (Qu F, 2022).

Increased fluctuation in HbA1c levels may suggest inadequate healthcare in the medical context, which is associated with inconsistent lifestyle changes and irregular adherence to medication. Healthcare practitioners should carefully evaluate factors such as individual behaviour, pharmaceutical reactions, and the presence of other medical diseases, as these can also influence the variance of HbA1c values (Ceriello A, 2019), (Osborn CY, 2016).

Ma Q, et al. discovered that HbA1c levels increased progressively with age in various gender groups. Therefore, there exists a link between HbA1c levels and age and gender within Chinese people. The association between HbA1c levels and future diabetes screening should be considered (Ma Q, 2016). Male participants in the age range of 30 to 59 years exhibited significantly elevated HbA1c levels in comparison to females ($P<0.05$). The likely reason for this phenomena is the inadequate control of blood pressure and blood lipids in males of this age group, whereas women may be more prone to the effects of their physiological cycle (Ma Q, 2016). In line with previous studies (Yang YC, 1997), (Karar T, 2015), gender discrepancies were detected in the connection between HbA1c and age. This finding could be linked to reduced hemoglobin levels in menstruating women who have a higher rate of red blood cell turnover, as suggested before (Yang YC, 1997). Women in the peri- and post-menopausal age groups demonstrated a more significant increase in comparison to men.

As individuals progress in age, numerous alterations occur. Initially, the function of pancreatic islets gradually decreases. Furthermore, there is a steady decline in tissue sensitivity to insulin and insulin receptor function. Finally, the amount of muscle tissue steadily declines, leading to a decrease in the overall consumption of glucose. As a person ages, the combined effects of these factors cause blood glucose levels to increase gradually, leading to increased HbA1c values, especially in older individuals (Ma Q, 2016).

6. Conclusion

6.5 Conclusion of goal No. one

In summary, our results indicate that HbA1c levels can be estimated almost exactly at population level in women in the age range of 20 and 70 years, when common metabolic parameters including lipid profile, uric acid and CRP levels are measured and severe anemia and inflammation are excluded. Further investigation is required to identify those factors in women older than 70 years and in men that contribute to HbA1c levels.

6.6 Conclusion of goal No. two

Based on our study, we concluded that in addition to age, the MCV parameter, which indirectly characterizes the average lifespan of red blood cells, is closely related to HbA1c values. However, a close relationship does not mean a clinically significant effect. Therefore, in routine clinical practice, apart from cases with severe hematopoietic abnormalities, the value of MCV does not need to be taken into account during the interpretation of HbA1c.

6.7 Conclusion of goal No. three

Serum level of vitamin D is negatively correlated with HbA1c level both in males and females.

6.8 Conclusion of goal No. four

The age group of 60–69 years old had the largest percentage of both males and females who were not improved in the next visit, and it also had the highest percentage of all improved females across all age groups.

7. Summary

Background: Several observations suggest the presence of possible association between HbA1c levels and several demographic and clinical parameters.

Objectives: Several objectives were present in this work, we examined the possible correlation between HbA1c concentration and the concentrations of serum levels of uric acid, triglyceride, cholesterol, CRP, vitamin D, and the CBC indices. Moreover, we searched for the possible association between age and/or gender with HbA1c levels in single visit and in visit-to-visit.

Method: HbA1C results extracted from the electronic laboratory database of Semmelweis hospital, for patients consulting this hospital in Budapest/Hungary. The records of a total of 20,416 patients, consulting the Semmelweis hospital between 2008–2012 were recruited. The selected patients' ages ranged between 20 – ≥ 90 years old. In addition to HbA1C, the results of laboratory parameters including CRP, vitamin D, CBC count, total cholesterol, LDL- and HDL, triglyceride, and uric acid were also extracted for the patients. Additional group of data were also analyzed, it was including 193,165 individuals included 92,700 males and 100,465 females, these data included HbA1c results for more than one visit for each patient with different ages and both genders.

Results: The developed formulas are as follow: HbA1c (estimated) in women = 0.752 + 0.237*log10(HDL/ cholesterol) + 0.156*log10 (cholesterol) + 0.077*log10 (triglyceride) + 0.025*log10(CRP) +0.001*log10 (age) − 0.026*log10(HDL/LDL) − 0.063*log10 (uric acid)-0.075*log10 (LDL)-0.199*log10(HDL); HbA1c (estimated) in men = 1.146 + 0.08*log10 (triglyceride) + 0.046*log10(CRP) + 0.01*log10 (cholesterol) + 0.001*log10 (age) − 0.014*log10(HDL)-0.018*log10(HDL/LDL)-0.025*log10(HDL/cholesterol) − 0.068*log10 (LDL)- 0.159*log10 (uric acid). MCV in both sexes, RDW in men negative, in women RBC showed a positive relationship with HbA1c. Based on the CE values, one of the tested parameters does not have a greater effect on HbA1c than age. Recorded in MCV, RDW, HB discrete ranges histogram is the same. The current study showed a weak negative correlation (R= – 0.14) between HbA1c and serum vitamin D levels among study group. Among all age groups, the females in age group 60-69 years old had the highest number of patients (n=2232) with HbA1c in diabetic range which also had the highest percentage of both not improved (20.8%) and improved (24.2%) HbA1c readings in the second visit; comparable values were also reported for the males.

Conclusion: HbA1c levels can be estimated almost exactly based on lipid profile, CRP and uric acid levels in female patients between 20 and 70 years. vitamin D is negatively correlated with HbA1c in both males and females. The age group of 60–69 years old had the largest percentage of both males and females who were not improved in the next visit.

8. References

Aguilar D, Bozkurt B, Ramasubbu K, Deswal A. Relationship of hemoglobin A1C and mortality in heart failure patients with diabetes. *J Am Coll Cardiol.* 2009; 54(5), pp. 422-428.

Al-Shaheeb S, Hashim HK, Mohammed AK, Almashhadani HA, Al Fandi A. Assessment of lipid profile with HbA1c in type 2 diabetic Iraqi patients. *Revista Bionatura,* 2020; 7, pp. 29-33.

Alaa AF, Ahmed KY. Correlation of Glycated Hemoglobin (Hba1c) and Serum Uric Acid in Type-2 Diabetic Patients. *Indian Journal of Public Health Research & Development,* 2019; 10(5), pp. 104-108.

Aldasouqi SA, Gossain VV. Hemoglobin A1c: past, present and future. *Ann Saudi Med.* 2008; 28(6), pp. 411-419.

Alqahtani R, Ebtehag FA. The Association Between Glycated Hemoglobin (HbA1c) Level and Vitamin D Level in Diabetes Mellitus Patients: A Cross-Sectional Study. *Cureus* . 2023; *15(10): e47166,* 15(10), p. e47166.

Al-Shoumer KA, Al-Essa TM. Is there a relationship between vitamin D with insulin resistance and diabetes mellitus? *World J Diabetes*. 2015; 6(8), pp.1057–1064.

Alzahrani SH, Baig M, Aashi MM, Al-Shaibi FK, Alqarni DA, Bakhamees WH. Association between glycated hemoglobin (HbA1c) and the lipid profile in patients with type 2 diabetes mellitus at a tertiary care hospital: a retrospective study. *Diabetes Metab Syndr Obes.* 2019; 12, pp. 1639-1644.

Arnetz BB, Kallner A, Theorell T. The influence of aging on hemoglobin A1c (HbA1c). *J Gerontol.* 1982; 37, p. 648–650.

Bae JC, Suh S, Jin SM, Kim SW, Hur KY, Kim JH, Min YK, Lee MS, Lee MK, Jeon WS, Lee WY, Kim KW. Hemoglobin A1c values are affected by hemoglobin level and gender in non-anemic Koreans. *J Diabetes Investig. ,* 2014; 5(1), pp. 60-65.

Bahceci, M, Tuzcu A, Ogun C, Iltimur K, Aslan C. Is serum C-reactive protein concentration correlated with HbA1c and insulin resistance in Type 2 diabetic men with or without coronary heart disease?. *J Endocrinol Invest* , 2005; 28, p. 145–150.

Bardini G, Rotella, CM, Giannini S. Dyslipidemia and diabetes: reciprocal impact of impaired lipid metabolism and beta-cell dysfunction on micro- and macrovascular complications. *Rev Diabet Stud.* 2012; 9, pp. 82-93.

Begum A, Irfan SR, Hoque MR, Habib SH, Parvin S, Malek R, Akhter S, Sattar S, Sarkar S. Relationship between HbA1c and Lipid Profile Seen in Bangladeshi Type 2 Diabetes Mellitus Patients Attending BIRDEM Hospital: A Cross-Sectional Study. *Mymensingh Med J. ,* 2019; 28(1), pp. 91-95.

Bhutto AR, Abbasi AA, Abro AH. Correlation of Hemoglobin A1c with Red Cell Width Distribution and Other Parameters of Red Blood Cells in Type II Diabetes Mellitus. *Cureus,* 2019; 11(8), pp. e5533.

Bookchin RM, Gallop PM. Structure of haemoglobin A1c: nature of the N-terminal beta chain blocking group. *Biochem Biophys Res Commun.,* 1968; 32, pp. 86-93.

Brzozowska M, Lewiński A. Changes of androgens levels in menopausal women. *Prz Menopauzalny,* 2020; 19(4), pp. 151-154.

Bunn HF, Haney DN, Gabbay KH, Gallop PM. Further identification of the nature and linkage of the carbohydrate in haemoglobin A1c. *Biochem Biophys Res Commun,* 1975; 67, pp. 103-109.

Carrera T, Bonamusa L, Almirall L, Navarro JM. Should age and sex be taken into account in the determination of HbA1c reference range? *Diabetes Care.* 1998; 21, pp. 2193–2194.

Ceriello A, Rossi MC, De Cosmo S, Lucisano G, Pontremoli R, Fioretto P, Giorda C, Pacilli A, Viazzi F, Russo G, Nicolucci A. Overall quality of care predicts the variability of key risk factors for complications in type 2 diabetes: an observational, longitudinal retrospective study. *Diabetes Care ,* 2019; 42, pp. 514–519.

Chen J, Ge J, Zha M, Miao JJ, Sun ZL, Yu JY. Effects of Uric Acid-Lowering Treatment on Glycaemia: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne),* 2000; 11, pp. 577-589.

Cohen RM, Haggerty S, Herman WH. HbA1c for the diagnosis of diabetes and prediabetes: is it time for a mid-course correction? *J Clin Endocrinol Metab.* 2010; 95(12), pp. 5203-5206.

Committee., I. E. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care, 2009;* 32(7), pp. 1327-1334.

Cui Y, Bu H, Ma X, Zhao S, Li X, Lu S. The Relation between Serum Uric Acid and HbA1c Is Dependent upon Hyperinsulinemia in Patients with Newly Diagnosed Type 2 Diabetes Mellitus. *J Diabetes Res.* 2016; 2016, pp. 1-6.

Cook DG, Shaper AG, D S Thelle DS, Whitehead TP. Serum uric acid, serum glucose and diabetes: relationships in a population study. *Postgraduate Medical Journal*, 1986; 62(733), pp. 1001-1006.

Çiçek B, Okuturlar Y, Deniz OC, Okuturlar Ö, Celik O, Serteser M, Şahin S. HbA1c and Lipid Profile Indices: Useful for cardiovascular risk prediction in non-diabetics? Endocrine Abstracts. 2023; 90, pp. EP1107.

Dakhale GN, Chaudhari HV, Shrivastava M. Supplementation of vitamin C reduces blood glucose and improves glycosylated hemoglobin in type 2 diabetes mellitus: a randomized, double-blind study. *Adv Pharmacol Sci.* ,2011; 2011, pp. 195271.

Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue.. *Diabetes Res Clin Pract.* , 2011; 93(1), pp. S52-529.

Eyth E, Roopa Naik R. Hemoglobin A1C. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.

Ford ES, COWIE CC, Chaoyang LI. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. *J Diabetes* , 2011; 3, pp. 67-73.

Frank MJ. Gender differences in glucose homeostasis and diabetes. *Physiol Behav.* , 2018; 187, pp. 20-23.

Farah J, Husan A, Farha A, Wasti AZ. Hyperglycemic induced variations in Hematological indices in type 2 diabetics. *Int J Adv Res* , 2013; 1, pp. 322-334.

Farahmand MA, Daneshzad E, Fung TT. What is the impact of vitamin D supplementation on glycemic control in people with type-2 diabetes: a systematic review and meta-analysis of randomized controlled trails. *BMC Endocr Disord*, 2023, 23(1), pp. 15.

Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence. *Clin Biochem Rev.*, 2013; 34(2), pp. 75-83.

Ford ES, Choi HK. Haemoglobin A1c, fasting glucose, serum C-peptide and insulin resistance in relation to serum uric acid levels—The Third National Health and Nutrition Examination Survey. *Rheumatology*, 2008; 47(5), pp. 713-717.

Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci.* , 2020; 21(17), pp. 6275.

Galvács H, Szabó J, Balogh Z. Risk-based diabetes screening in a Hungarian general practice: comparison of laboratory methods and diagnostic criteria. *Prim Health Care Res Dev.,* 2021; 22, p. e17.

Gautam D, Adhikari S, Thapa R. Study to determine between HbA1C and C-reactive protein in Diabetes mellitus. *Journal of Pathology of Nepal,* 2023; 13(1), pp. 1979–1982.

Gherghina M-E, Peride I, Tiglis M , Neagu TP, Niculae A, Checherita IA. Uric Acid and Oxidative Stress—Relationship with Cardiovascular, Metabolic, and Renal Impairment. *International Journal of Molecular Sciences,* 2022; 23(6), p. 3188.

Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* , 2010; 107(9), pp. 1058-1070.

Gülsen Ş, Kahvecioğlu ED, Başak C, Gümüş A, Beyazıt SY, Evran B. The effect of age and gender on HbA1c levels in adults without diabetes mellitus. *J Med Biochem.* , 2023; 42(4), pp. 714-721.

Gupta UK, Mohapatra TK, Potdar P. Correlation between hs-CRP, HbA1c and Oxidative Stress in Type-2 Diabetic Patients. *Asian Journal of Medical Research*, 2019; 7(4), pp. BC01-BC06.

Habeeb AM, Al-Sarray AA**,** Al Hafidh AH. Lipid profiles and HBA1c level Among in Type, 2 Diabetes patients in Hilla city, Iraq. *Journal of Population Therapeutics and Clinical Pharmacology, 30(7), 273–278*, 2023; 30(7), pp. 273–278.

Halcox JPJ, Roy C, Tubach F, Banegas JR, Dallongeville J, De Backer G, Guallar E, Sazova O, Medina J, Perk J, Steg PG, Rodríguez-Artalejo F, Borghi C. C-reactive protein levels in patients at cardiovascular risk: EURIKA study. *BMC Cardiovasc Disord.,* 2014; 14, p. 25.

Hardikar PS, Joshi SM, Bhat DS, Raut DA, Katre PA, Lubree HG, Jere A, Pandit AN, Fall CHD, Yajnik CS. Spuriously high prevalence of prediabetes diagnosed by HbA1c in young Indians partly explained by hematological factors and iron deficiency anemia. *Diabetes Care,* 2012; 35, p. 797–802.

Hashimoto Y, Futamura A, Ikushima M. Effect of aging on HbA1c in a working male Japanese population. *Diabetes Care,* 1995; 18, p. 1337–1340.

HN, G., 1991. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis.. *Diabetes Care*, 14(9), pp. 839-855.

Huang R, Yan L, Lei Y. The relationship between high-density lipoprotein cholesterol (HDL-C) and glycosylated hemoglobin in diabetic patients aged 20 or above: a cross-sectional study. *BMC Endocr Disord.* ,2021; 21(1), p. 198.

Huang SH, Huang PJ, Li JY. Hemoglobin A1c levels associated with age and gender in taiwanese adults without prior diagnosis with diabetes. *Int. J. Environ. Res. Public Health.* 2021; 18, p. 3390.

Huang Z, Liu Y, Mao Y, Yanfang Mao, Chen W, Xiao Z, Yuet Y. Relationship between glycatedhaemoglobin concentration and erythrocyte survival in type 2 diabetes mellitus determined by a modified carbon monoxide breath test. *J Breath Res*, 2018; 12, p. 026004.

Huisman TH, Martis EA, Dozy A. Chromatography of hemoglobin types on carboxymethylcellulose. *J Lab Clin Med.* 1957; 52, pp. 312-27.

Hussain A, Ali I, Ijaz M, Rahim A. Correlation between hemoglobin A1c and serum lipid profile in Afghani patients with type 2 diabetes: hemoglobin A1c prognosticates dyslipidemia. *Ther Adv Endocrinol Metab.* 2017; 8(4), pp. 51-57.

Kilpatrick ES. Haemoglobin A1c in the diagnosis and monitoring of diabetes mellitus. J Clin Pathol. , 2008; 62(9), pp. 977–82.

Kushner JA. The role of aging upon β cell turnover.. *J Clin Invest.* , 2013; 123(3), pp. 990-995.

Kalairajan S, Durairaj K, Malathy AR. A study on influence of iron deficiency anaemia over HbA1c levels. *Int J Adv Med.* 2019; 6, p. 2349–3933.

Karar T, Alhammad RIS, Fattah MA, Alanazi A, Qureshi S. Relation between glycosylated hemoglobin and lipid and thyroid hormone among patients with type 2 diabetes mellitus at King Abdulaziz Medical City, Riyadh. *J Nat Sci Biol Med. ,2*015; 6(1), p. S75.

Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. *Clin. Exp. Med.* 2007; 7, pp. 24- 29.

Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. *N Engl J Med.* 1976; 295, pp. 417-420.

Koga M, Morita S, Saito H, Kasayama S. Association of erythrocyte indices with glycated haemoglobin in premenopausal women. *Diabet Med* , 2007; 24, pp. 843-847.

Kosmas CE, Silverio D, Sourlas A, Garcia F, Montan PD, Guzman E. Impact of lipid-lowering therapy on glycaemic control and the risk for new-onset diabetes mellitus. *Drugs Context.* 2018; 7, p. 212562.

Lai Y, Lin Z, Zhu Z. Association between hemoglobin within the normal range and hemoglobin A1c among Chinese non-diabetes adults. *BMC Endocr Disord* , 2021; 35-42.

Lee HS. Sex Difference in the Effect of Body Mass Index and Stress on High-Risk Diabetes Mellitus in Korean Adults. *J. Korean Biol. Nurs. Sci.* 2019; 21, p. 224–230.

Lee PG, Halter JB. The Pathophysiology of Hyperglycemia in Older Adults: Clinical Considerations. *Diabetes Care,*2017; 40(4), pp. 444-452.

Li D, Zhong J, Zhang Q, Zhang J. Effects of anti-inflammatory therapies on glycaemic control in type 2 diabetes mellitus. *Front Immunol.* 2023; 14, pp. 1125116.

Lind M, Imberg H, Coleman RL, Nerman O, Holman RR. Historical HbA1c Values May Explain the Type 2 Diabetes Legacy Effect: UKPDS 88. *Diabetic Care* , 2021; 44(10), pp. 2231-2237.

Lippi G, Targher G, Salvagno GL, Guidi GC. Increased red blood cell distribution width (RDW) is associated with higher glycosylated hemoglobin (HbA1c) in the elderly. *Clin Lab.* 2014; 60(12), pp. 2095-2098.

Ma QL, Liu HM, Xiang GX, Shan WS, Xing WL. Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus. *Biomed Rep.* 2016; 4(6), pp. 737-740.

McPherson JD, Shilton BH, Walton DJ. Role of fructose in glycation and cross-linking of proteins. *Biochemistry* , 1988; 27 (6), p. 1901–1907.

Meriga RK, Vishnu Anjan Nareddy VA, Bhavya SV, Sreekeerthi C. Correlation between glycemic control, lipid profile and C-reactive protein in adults with type 2 diabetes mellitus done in a tertiary care hospital of Nellore, India. *Int J Adv Med* , 2020; 7, pp. 1312-1317.

Meshram A, Agrawal U, Dhok A, Adole P, Meshram K, Khare R. HbA1c, hs-CRP and anthropometric parameters evaluation in the patients of diabetes mellitus of central rural

India. *International Journal of Medical Science and Public Health,* 2013; 2(2), pp. 293-296.

Miedema K. Standardization of HbA1c and Optimal Range of Monitoring. *Scandinavian Journal of Clinical and Laboratory Investigation,* 2005; 240, pp. 61-72.

Mitri J, Pittas AG. Vitamin D and diabetes. *Endocrinol Metab Clin North Am. ,* 2014; 43(1), p. 205–232.

MS Radin. Pitfalls in hemoglobin A1c measurement: when results may be misleading. *J Gen Intern Med. ,* 2014; 29(2), pp. 388-94.

Muhammad FH, Syafril S, Lindarto D. Elevated uric acid level decreases glycated hemoglobin in type 2 diabetes mellitus. *Univ Med.,* 2014; 33(3), pp. 199-204.

Muhammed AH. Correlation between HbA1c and lipid profile in patients with Type2 diabetes mellitus. *Kirkuk Journal of Medical Sciences ,* 2023; 11(1); 101-107.

Nasir NM, Thevarajah M, Yean CY. Hemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. *Int J Diabetes Dev Ctries.* 2010; 30(2), pp. 86-90.

Niwaha AJ, Rodgers LR, Greiner R, Balungi PA, Mwebaze R, McDonald TJ, Hattersley AT, Shields BM, Nyirenda MJ, Jones AG. HbA1c performs well in monitoring glucose control even in populations with high prevalence of medical conditions that may alter its reliability: the OPTIMAL observational multicenter study. *BMJ Open Diabetes Res Care*, 2021; 9(1), p. e002350.

Nnakenyi ID, Nnakenyi EF, Parker EJ, Uchendu NO, Anaduaka EG, Ezeanyika LU. Relationship between glycaemic control and lipid profile in type 2 diabetes mellitus patients in a low-resource setting. *Pan Afr Med J.* 2022; 41, p. 281.

Nutall FQ. Effect of age on the % of hemoglobin A1c and the % of total glycohemoglobin in non-diabetic persons. J Lab Clin Med.1999; Volume 134, p. 451–453.

Omodanisi EI, Tomose Y, Okeleye BI, Ntwampe SKO, Aboua YG. Prevalence of Dyslipidaemia among Type 2 Diabetes Mellitus Patients in the Western Cape, South Africa. *Int J Environ Res Public Health.* 2020; 17(23), p. 8735.

Osborn CY, Mayberry LS, Kim JM. Medication adherence may be more important than other behaviours for optimizing glycaemic control among low-income adults. *J Clin Pharm Ther,* 2016; 41, p. 256–259.

Park S, Kim DS, Kang S. Vitamin D deficiency impairs glucose-stimulated insulin secretion and increases insulin resistance by reducing PPAR-γ expression in nonobese type 2 diabetic rats. *J Nutr Biochem.* 2016; 27, pp. 257-265.

Pfützner A, Schöndorf T, Hanefeld M, T Forst. High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone. *J Diabetes Sci Technol.* 2010; 4(3), p. 70.

Pittas AG, Dawson-Hughes B. Vitamin D and diabetes. *J Steroid Biochem Mol Biol.* 2010; 121(1-2), p. 425–429.

Pittas AG, Lau J, Hu FB, Hughes BD. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2007; 92(6), p. 2017–2029.

Pongudom S, Chinthammitr Y. Determination of Normal HbA1C Levels in Non-Diabetic Patients with Hemoglobin E. *Annals of Clinical & Laboratory Science,* 2019; 49(6), pp. 804-809.

Qu F, Shi Q, Wang Y, Shen Y, Zhou K, Pearson ER. Visit-to-visit glycated hemoglobin A1c variability in adults with type 2 diabetes: a systematic review and meta-analysis. *Chin Med J (Engl).* 2022; 135(19), pp. 2294-2300.

Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Commun. ,* 1969; 36, pp. 838-843.

Rose AM, Tongate C, Valdes R Jr. A hemoglobin A1C immunoassay method not affected by carbamylated hemoglobin. *Ann Clin Lab Sci. ,* 1995; 25(1), pp. 13-9.

Roth J, Müller N, Lehmann T. HbA1c and age in non-diabetic subjects: An ignored association?. *Exp Clin Endocrinol Diabetes.* 2016; 124, p. 637–642.

Rusdiana, Widjaja, S, Syahputra M, Savira M. Association between Haemoglobin A1c and Uric Acid Levels among Patients with Diabetes Mellitus Type 2 at a Primary Health Care Clinic in North Sumatera, Indonesia. *Maced J Med Sci.,* 2018; 6(9), pp. 1633-1635.

Salimon AH, Patil HA. Correlation of red blood cell distribution width (RDW) and hemoglobin A1C (HbA1C) levels in diabetic individuals. *nt J Innov Res Sci Eng Technol.* 2017; 6, p. 8227–8239.

Sarinnapakorn V, Wanicagool W. Association between hs-CRP and Hba1c in overweight type 2 diabetic female patients. *Journal of the Medical Association of Thailand,* 2013; 96(3), pp. 554-558.

Seo YH, Shin HY. Relationship between hs-CRP and HbA1c in Diabetes Mellitus Patients: 2015-2017 Korean National Health and Nutrition Examination Survey. *Chonnam Med J.* 2021; 57(1), pp. 62-67 .

Shanmuga P, Nasreen B. Correlation of Lipid Profile with Duration of Diabetes and HbA1c Levels in Type 2 Diabetes Mellitus Patients: A Descriptive Cross-sectional Study. *SBV Journal of Basic, Clinical and Applied Health Science,*2020; 3(1).

Shen Q, He TC, Li T, Szeto IMY, Mao S, Zhong WX, Li P, Jiang H, Zhang YM. Synergistic effects of overweight/obesity and high hemoglobin A1c status on elevated high-sensitivity C-reactive protein in Chinese adults: a cross-sectional study. *Front Nutr*. 2023; 10, p. 1156404.

Sherif H, Ramadan N, Radwan M, Hamdy E, Reda R. Red cell distribution width as a marker of inflammation in type 2 diabetes mellitus. *Life Sci J.* 2013; 10, pp. 1501-1507.

Shi CH, Wang C, Bai R, Zhang XY, Men LL, Du JL. Associations among glycemic excursions, glycated hemoglobin and high-sensitivity C-reactive protein in patients with poorly controlled type 2 diabetes mellitus. *Experimental and Therapeutic Medicine,* 2015; pp. 1937-1942.

Sproston NR, Ashworth, JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol. ,* 2018; 9, pp. 754.

Strandberg TE, Tilvis RS. C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. *Arterioscler Thromb Vasc Biol.* 2000; 20(4), pp. 1057-1060.

Stratton MI, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden DH, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): Prospective observational study. BMJ, 2000; 321, p. 405–412.

Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: A high-risk state for diabetes development. *Lancet,* 2012; 379 (9833): p. 2279–2290.

Temelkova-Kurktschiev T, Siegert G, Bergmann S, Henkel E, Koehler C, Jaross W, Hanefeld M. Subclinical inflammation is strongly related to insulin resistance but not to

impaired insulin secretion in a high risk population for diabetes. *Metabolism.* 2002; 51, pp. 743–749.

Teo LM, Lim WY, Ke Y, Sia IKL, Gui CH, Abdullah H R. A prospective observational prevalence study of elevated HbA1c among elective surgical patients. *Sci Rep. ,* 2020; 10(1), p. 19067.

Veeranna V, Zalawadiya SK, Panaich SS. The association of red cell distribution width with glycated hemoglobin among healthy adults without diabetes mellitus. *Cardiology ,* 2012; 122, pp. 129-132.

Vinod A. Relationship between serum high-sensitivity C-reactive protein (hs-CRP) and glycated hemoglobin (HbA1c) in South Indian population. *Biomedicine.* 2023; 43(1), pp. 310-316.

Wei FJ, Chang BC, Yang XL, Wang YG, Chen LM, Li WD. Serum Uric Acid Levels were Dynamically Coupled with Hemoglobin A1c in the Development of Type 2 Diabetes. *Sci Rep* , 2016; 6, pp. 28549.

Weykamp C. HbA1c: a review of analytical and clinical aspects. *Ann Lab Med.,* 2013; 33(6), pp. 393-400.

Whitehead TP, Jungner I, Robinson D, Kolar W, Pearl A, Hale A. Serum urate, serum glucose and diabetes. *Annals of Clinical Biochemistry,* 1992; 29(2), pp. 159-161.

WHO, 2011. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. *Abbreviated Report of a WHO Consultation. Geneva: ; 2011. 2, Glycated haemoglobin (HbA1c) for the diagnosis of diabetes.*

William P F. Linking inflammation and thrombosis: Role of C-reactive protein. *World J Cardiol.* 2010; 2(11), pp. 365-369.

Wu L, Lin H, Gao J. Effect of age on the diagnostic efficiency of HbA1c for diabetes in a Chinese middle-aged and elderly population: The Shanghai Changfeng Study. *PLoS One,* 2017; 12(9), p. e018460.

Xiong Q, Liu J, Xu Y. Effects of Uric Acid on Diabetes Mellitus and Its Chronic Complications. *Int J Endocrinol.* 2019; 2019, pp. 9691345.

Yang YC, Lu FH, Wu JS, Chang CJ. Age and sex effects on HbA1c. A study in a healthy Chinese population.. *Diabetes Care.*1997; 20, p. 988–991.

Yong PH, Weinberg L, Torkamani N, Churilov L, Robbins RJ, Ma R, Bellomo R, Lam QT, Burns JD, Hart GK, Lew JF, Mårtensson J, Story D, Motley AN, Johnson D, Zajac

JD, Ekinc EI. The presence of diabetes and higher HbA1c Are independently associated with adverse outcomes after surgery. *Diabetes Care*, 2018; 41, p. 1172–1179.

9. Bibliography

9.1 Publications on which the dissertation is based

1. Jalal DA, Vásárhelyi B, Blaha B, Tóth Z, Szabó TG, Gyarmati B. Interrelationship of hemoglobin A1c level lipid profile, uric acid, C-reactive protein levels and age in a large hospital database. Mol Cell Probes. 2023 Dec;72:101933..
2. Vásárhelyi B, Dlovan AJ, Blaha B, Tóth Z, Szabó GT, Gyarmati B. Vörösvérsejt-paraméterek és a hemoglobin-A1c (HbA1c)-szint kapcsolata 10 év adatainak retrospektív értékelése alapján [Relationship between red blood cell parameters and hemoglobin A1c levels based on a retrospective evaluation of 10 years of data]. Orv Hetil. 2024 Feb 18;165(7):243-248.

9.2 Additional publications

1. Tóth Z, Szalay B, Gyarmati B, Jalal DA, Vásárhelyi B, Szabó T. Vitamin D Deficiency has no Impact on PSA Reference Ranges in a General University Hospital – A Retrospective Analysis. EJIFCC. 2020 Sep 29;31(3):225-230. PMID: 33061877; PMCID: PMC7545131.
2. Toth Z, Szalay B, Gyarmati B, Jalal DA, Vasarhelyi B, Szabo T. Prostate specific antigen serum levels in patients with different levels of hepatic or renal impairment and in those with systemic inflammation in a university hospital. A retrospective analysis of 10 years of laboratory data. Open Access J Urol Nephrol 2020, 5(3): 000184. doi: 10.23880/OAJUN-16000184.
3. Karvaly G, Kovács K, Gyarmati M, Gerszi D, Nagy S, Jalal DA, Tóth Z, Vasarhelyi B, Gyarmati B. Reference data on estrogen metabolome in healthy pregnancy. Mol Cell Probes. 2024 Mar 4;74:101953. doi: 10.1016/j.mcp.2024.101953. PMID: 38432490.

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