EXPLORING LABORATORY PARAMETERS THAT IMPACT HBA1C LEVELS

PhD thesis

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1. INTRODUCTION

1.1 Generation of glycated hemoglobin

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

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.

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.

1.2 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. Since the average lifetime of RBCs is normally 3-4 months, the test is representing this three-month period.

Diabetes Control and Complications Trial units 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. However, in clinical practice mmol/mol units are converted to per cent values for clinical decision making.

1.3 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.

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 . 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.

1.4 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. 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.

1.5 Clinical Significance of glycated hemoglobin

Blood loss, hemolytic anemia, and hypersplenism are common causes of alterations in HbA1c readings, 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.

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.

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.

1.6 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.

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). 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.

1.7 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. 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 plaque. The CRP amount is also associated to the rate of cardiovascular event-free survival.

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.

1.8 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. 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

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. 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.

The relation between HbA1c and erythrocytes parameters

1.8.1 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. 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. 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.

1.8.2 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. 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. RDW and HbA1c have been demonstrated to positively correlate in another study by Sherif et al., however statistically, the association was not significant.

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.

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. 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 .

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. 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.

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. Lower levels of 25(OH) D have been linked to a higher frequency of metabolic syndrome and diabetes mellitus.

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.

3. METHODS

3.1 Objective 1

Our laboratory database contains 200 million records of laboratory measurements from the period between 2008 and 2018. 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, in age group between 20 and 90 years of age. Finally, data of 9599 male and 10,817 female patients were used for the analysis. In order to assess the impact of gender, age, CRP, total cholesterol (along with HDL/LDL levels), triglyceride and uric acid levels on HbA1c %, a multiple linear regression model was applied. 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 Objective 2

We used the data of more than 60,000 patients for analysis. The relationship between red blood cell parameters (HB, HCT, MCV, RBC, RDW) and the HbA1c level were analyzed 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.

3.3 Objective 3

Data assembly was performed by capturing the results of HbA1c and serum vitamin D, from electronic health records of 9599 male and 10817 female patients visiting the Semmelweis hospital. The results represent the initial laboratory before the medical intervention. The relationship between the level of vitamin D and the HbA1c was investigated using the Pearson correlation coefficient.

3.4 Objective 4

The investigation employed data from 6069 males and 5998 females' participants' two visits' worth of HbA1c readings. The HbA1c results of the two visits were compared in regards of age and gender. If the expected cells included were less than 5, then Fisher's exact test was used instead of Chi-square test with adopting a significance level of 5% ($p<0.05$).

4 RESULTS

4.1 Results of objective 1

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.

4.2 Results of objective 2

There was 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), while for HbA1c levels and MCV revealed a negative weak correlation (R= – 0 .1) though still significant correlation ($p<2.2e^{-16}$). For Hemoglobin levels and HbA1c levels showed a weakly negative but nonetheless statistically significant (p2.2e-16) association. HbA1c% levels and hemoglobin under 100 g/L was measured and the model was not splitted by gender and results indicated a weak correlation (R value = 0.033) but statistically significant (p=0.018).

4.2 Results of objective 3

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); 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.

4.3 Results of objective 4

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 diabetic range. In the second HbA1c test for the same individuals, the normal range was recorded for 9,202 (33.7%), prediabetes range was recorded for 6,536 (23.9%), while diabetes range was measured in 11,554 (42.3%). The mean HbA1c reading in the first measurement was 6.9±2.02%, while for the second reading was 6.66±1.70 %. 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%).

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 visit when compared to those in the same age group with increased not no decline in HbA1c (14.76% vs 11.38 for age group 20-29 years; and 24.48% vs 23.32% for age group 70-79 years). all the other age groups had increased in %s of patients with increased HbA1c in the second visit when compared to the first one. 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 with lowered HbA1c in the second visit than those with increased HbA1c values in the same age groups; while the other three age groups (40-49 years, 50-59 years, and 60-69 years) had higher %s of individuals with increased HbA1c results in the second visit when compared to those with lowered HbA1c results.

4. 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 , 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, 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.

The results of Begum A, et al 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 . Al shaheeb, et al, assessed lipid profile with HbA1c in type 2 diabetic Iraqi patients and reported results in consistence with our findings .

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.

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. Alzahrani SH, et al. reported significant association of HbA1c% only with TG and not with other lipid parameters.

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, reported positive correlation between HbA1c and CRP in diabetic patients and in non-diabetics, 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 , Jeong Woo Seo and his colleague found that HbA1c is better correlated with CRP than fasting blood sugar . 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 .

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. 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, 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.

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 . The results of Alqahtani R. and her colleague were comparable to ours. 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.

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. Research in rodents and people has shown that aging can have a unique impact on b-cell regeneration and performance.

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.

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, in Singaporean study, the prevalence of diabetes mellitus in a hospital-based study population is 19%, 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% .

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. On the other hand, those with HbA1c levels below 6% had a decreased risk of long-term problems.

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.

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. 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 .

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.

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.

5. CONCLUSION

6.1 Conclusion of objective 1

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.

6.2 Conclusion of objective 2

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.

6.3 Conclusion of objective 3

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

6.4 Conclusion of objective 4

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.

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6. PUBLICATIONS

7.1 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.

7.2 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.