Journal of Clinical Laboratory Analysis

WILEY

RESEARCH ARTICLE OPEN ACCESS

Unlocking Optimal Glycemic Interpretation: Redefining HbA1c Analysis in Female Patients With Diabetes and Iron-Deficiency Anemia Using Machine Learning Algorithms

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Received: 13 March 2024 | **Revised:** 27 May 2024 | **Accepted:** 23 June 2024

Funding: The authors received no specific funding for this work.

Keywords: diabetes mellitus | hemoglobinA1c | iron deficiency anemia | machine learning

ABSTRACT

Objective: In response to the nuanced glycemic challenges faced by women with iron deficiency anemia (IDA) associated with diabetes, this study uses advanced machine learning algorithms to redefine hemoglobin (Hb)A1c measurement values. We aimed to improve the accuracy of glycemic interpretation by recognizing the critical interaction between erythrocytes, iron, and glycemic levels in this specific demographic group.

Methods: This retrospective observational study included 17,526 adult women with HbA1c levels recorded from 2017 to 2022. Samples were classified as diabetic, prediabetic, or non-diabetic based on HbA1c and fasting blood glucose (FBG) levels for distribution analysis without impacting model training. Support Vector Machines, Linear Regression, Random Forest, and K-Nearest Neighbor algorithms as machine learning (ML) methods were used to predict HbA1c levels. Following the training of the model, HbA1c values were predicted for the IDA samples using the trained model.

Results: According to our results, there has been a 0.1 unit change in HbA1c values, which has resulted in a clinical decision change in some patients.

Discussion: Using ML to analyze HbA1c results in women with IDA may unveil distinctions among patients whose HbA1c values hover near critical medical decision thresholds. This intersection of technology and laboratory science holds promise for enhancing precision in medical decision-making processes.

1 | Introduction

Hemoglobin (Hb) A1c is formed by non-enzymatic saccharification of Hb A in the formation of Hb erythrocytes. HbA1c is one of the diagnostic criteria for diabetes established by the American Diabetes Association (ADA). What makes this parameter even more valuable is that it provides information about the patient's blood glucose regulation over the past 2–3 months [1]. However, there are problems in using the ADA diagnostic criteria for HbA1c in anemic patients [2]. When used to control blood sugar in patients with diabetes, it is important to realize that HbA1c levels may be affected by anemia [3–6].

Therefore, HbA1c can lead to false results and misinterpretations in anemic patients. In 2021, the global prevalence of anemia was recorded at 1.92 billion people, an increase of 420 million

cases over 30 years [7]. Nearly 29.9% (95% uncertainty interval: 27.0%–32.8%) of women aged 15–49 years worldwide are anemic, according to the World Health Organization. In 2019, there were more than half a billion people in this age group. While the prevalence among non-pregnant women of reproductive age is the same, about 29.6%, the prevalence among pregnant women is higher, about 36.5%. Iron deficiency anemia (IDA) is the main cause of anemia in women in this age group [8].

Previous studies have shown the effect of IDA on HbA1c values [9, 10]. A recently completed prospective study also provided evidence that IDA is associated with increased HbA1c levels and that HbA1c levels significantly decrease after iron treatment [2]. IDA should be considered before making any decisions about diagnosis or treatment based on HbA1c. In people with IDA, HbA1c levels may cause an overestimation of blood sugar status [11, 12].

In anemic patients, non-enzymatic glycation products such as fructosamine (F) and glycated albumin (GA) have been suggested as alternatives to HbA1c for long-term monitoring of blood glucose levels, providing clinicians valuable insights [13]. F and GA are formed by the glycation of circulating albumin, globulins, and lipoproteins. Although serum F is positively correlated with HbA1c, it has demonstrated superior predictive ability for hyperglycemia in ROC analyses, likely because it reflects a longer time interval [14].

Despite recent studies attempting to establish predictive values for F in the development of microvascular complications in diabetes [15], the fact that F reflects blood glucose regulation over a shorter period (approximately the last 2 weeks), its analyses are not as standardized as HbA1c, and most importantly, the absence of direct evidence-based targets for F and GA levels make clinical interpretation challenging. Research investigating the usability of F in place of HbA1c has shown that the correlation between HbA1c and blood glucose concentrations remains stronger, reinforcing HbA1c as the most valid method for monitoring long-term blood glucose regulation monitoring [16].

Diabetes is a common disease in society, and its prevalence is steadily increasing. The number of individuals with diabetes worldwide is projected to reach 629 million by 2045 [13]. It is crucial to address the existing gaps and deficiencies in the management of this increasingly prevalent disease.

Therefore, knowing the correct HbA1c levels in women with IDA is essential for diagnosing, monitoring, and managing diabetes. One possible tactic is to use a machine learning-based method to create different models that consider individual characteristics, iron levels, and other clinical parameters [17, 18].

This study aimed to evaluate and improve glycemic control in women with IDA and diabetes. Our objective is to enhance the clinical management of patients who could be misdirected because of the complex relationship between IDA and diabetes by using these algorithms.

To achieve this, data from both IDA and non-anemic patients were collected (for details see Section 2.3). The non-anemic data were divided into training and test datasets. The training data were used to train a machine learning model for predicting HbA1c (for details on the prediction model, see Section 2.4). The prediction errors of the trained model were evaluated on the test data using various metrics (for details, see Section 2.5), and the results were reported.

The rest of the paper is organized as follows: Section 2 details the materials and methods used in this study. This section begins with a description of the study design and the characteristics of the participants. This is followed by an outline of the criteria and the process for participant selection. The biochemical analysis methods are then thoroughly explained. Next, the ethical considerations pertinent to this study are discussed. The section concludes with an overview of the HbA1c prediction machine learning methods and their implementation. Section 3 reports the findings of our study. This is followed by a comprehensive discussion in Section 4, which interprets the findings and places them within the context of existing literature. Section 5 addresses the limitations of our study and suggests directions for future research. Finally, Section 6 summarizes the key findings and their implications.

2 | Materials and Methods

2.1 | Study Design and Participants

The retrospective observational cohort study included 17,526 women aged 18 years with documented HbA1c levels between 2017 and 2022. The data set was obtained from the laboratory information system of Kocaeli University Hospital [fasting blood glucose (FBG), HbA1c, total iron binding capacity (TDBC), unsaturated iron binding capacity (UIBC), iron, transferrin saturation (TS), ferritin, hematocrit (htc), Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell (RBC)].

All parameters were obtained simultaneously and as a single result for each participant. The study consisted of two groups of patients with IDA and non-anemic (NA) female patients. Diabetic (FBG ≥126 mg/dL and HbA1c ≥6.5%), prediabetic (100 mg/dL ≤ FBG <126 mg/dL and 5.7 ≤ HbA1c <6.5%), and non-diabetic (FBG <100 mg/dL and HbA1c <5.7%) groups were formed as subgroups of these groups according to FBG and HbA1c in each group [19].

The IDA group consisted of participants with hemoglobin levels <12 g/dL, MCV < 80 fL [20], and MCH 28 pg/cell or less [21]. To differentiate microcytic anemia other than IDA, those with TS 16% and serum ferritin 30 ng/mL were included in the study [20]. Participants with Hb levels between 12.0 and 15.0 g/dL, MCV between 80 and 100 fL [22], and MCH > 28 pg/cell were considered NA [21]. Participants under the age of 18 years and those with missing or incomplete data that would undermine the validity of the study were excluded.

The International Hypoglycemia Study Group suggested that ≤70 mg/dL (3.9 mmol/L) should be used as a hypoglycemia warning value (level 1) and <54 mg/dL (<3.0 mmol/L) should be used as a clinically significant hypoglycemia level (level 2) in the classification of hypoglycemia in diabetic patients. These limits are especially important in the treatment management of diabetic patients [23]. Considering that each laboratory may have different measurement uncertainties, patients with FBG levels

below 60 mg/dL were not included in our study. We decided to use this cutoff because there may be patients with diabetes that are likely to have low HBA1c levels because of the possibility of having frequent hypoglycemic episodes.

In the diabetic patient group, the results of patients who did not meet both criteria for both FBG and Hb A1c were not included in the study. Among patients with diabetes, those with Hb A1c values between 6.5 and 9 were included in the study. In particular, it was thought that it would be appropriate to use these intervals to report changes that may occur at the Clinical decision boundary point. The final number of remaining samples is given in Table 1.

2.2 | Biochemical Analysis

FBG, TIBC, UIBC, Fe, TS, and ferritin were analyzed using a Cobas 8000 e 801 module (Roche Diagnostics GmbH, Mannheim, Germany) modular immunochemistry (IC) AutoAnalyzer. A Sysmex XN-10 automatic hematology analyzer (Sysmex CorporationTM, Kobe, Japan) was used for complete blood count analysis (CBC), and Hb, MCH, MCHC, MCV, RBC, and HCT parameters were selected from CBC in our study. HbA1c was measured using Liforofic H9 with high-performance liquid chromatography (HPLC) method (Lifotronic Technology, Shenzhen, China). At least two levels of routine internal quality control were performed daily for all parameters. External quality controls were performed monthly.

2.3 | Ethical Considerations

This study was approved by the ethics committee of our hospital with project number GOKAEK-11.24. 2023/192.

2.4 | Prediction Methods

The prediction of HbA1c levels has been addressed using various machine learning methods, including Support Vector Machines (SVM), Linear Regression (LR), Random Forest (RF), and K-Nearest Neighbor (KNN).

SVMs are versatile in both classification and regression tasks, with Support Vector Regression (SVR) specifically applied to regression problems. SVR aims to find the best-fitting line within an ε-insensitive tube in a high-dimensional feature space [24, 25].

LR is a statistical method that models the relationship between a dependent variable and an independent variable. It seeks the best-fitting linear equation to predict the dependent variable based on the independent variables [26, 27].

RFR is a robust machine learning method that combines multiple decision trees to create a comprehensive model. Each tree depends on a random vector sampled independently for all trees in the forest [28, 29].

KNN is a nonparametric algorithm for predicting continuous outcomes. It operates on the principle that similar data points have similar output values. The KNN identifies the k-nearest neighbors in the training dataset using a chosen distance metric [30].

TABLE 1 | The dataset details used in experiments.

Diabetes	IDA	NA
Non-diabetic	1116	10,527
Prediabetic	346	2749
Diabetic	406	2382

Abbreviations: IDA, ıron deficiency anemia; NA, non-anemic.

2.5 | Implementation of the Machine Learning Algorithms

The dataset is divided into two subsets, namely NA and IDA. The NA dataset is randomly partitioned into training and testing sets at an 80–20 ratio. The training data were used to train a machine learning model for predicting HbA1c. The prediction errors of the trained model were evaluated on the test data (which the HbA1c is not included) using various metrics, and the results were reported. Prediction errors, quantified through mean absolute error (MAE), mean squared error (MSE), root mean squared error (RMSE), and R square (R^2) metrics, are computed for the test set in relation to the prediction models. The Process of separating the dataset, training the prediction model, and conducting testing is delineated in the flowchart presented in Figure 1.

Subsequently, the laboratory-measured HbA1c values for IDA patients were compared with the HbA1c predictions obtained by inputting the IDA parameters into the model trained on non-anemic data. These procedural steps are visually presented in Figure 2. We hypothesized that the difference between the HbA1c prediction made by the model trained on non-anemic data and the laboratory-measured HbA1c for an IDA patient could be used as a correction parameter. Using this correction parameter, we attempted to identify the optimal glycemic interpretation for IDA patients.

3 | Results

In the experimental investigations, the SVM, LR, RFR, and KNN prediction methods (see Section 4. for details) were implemented. To assess these methods, MAE, MSE, RMSE, and R^2 metrics were employed. The prediction models were trained using the NA part of the dataset outlined in Table 1. After the entire dataset was divided into two subgroups (IDA, NA), the feature values were normalized before being used in model training and testing. The test results for each trained model are given in tables (Tables 2 and 3). In addition, the results in the tables are visualized as bar graphs (Figures 3 and 4) for easier interpretation. The implementation steps of the experimental studies are visually shown in Figures 1 and 2.

Upon examining the results provided in Table 2 and Figure 3, it is evident that the models trained with NA data yielded the best outcome with the implementation of the RFR method. Among

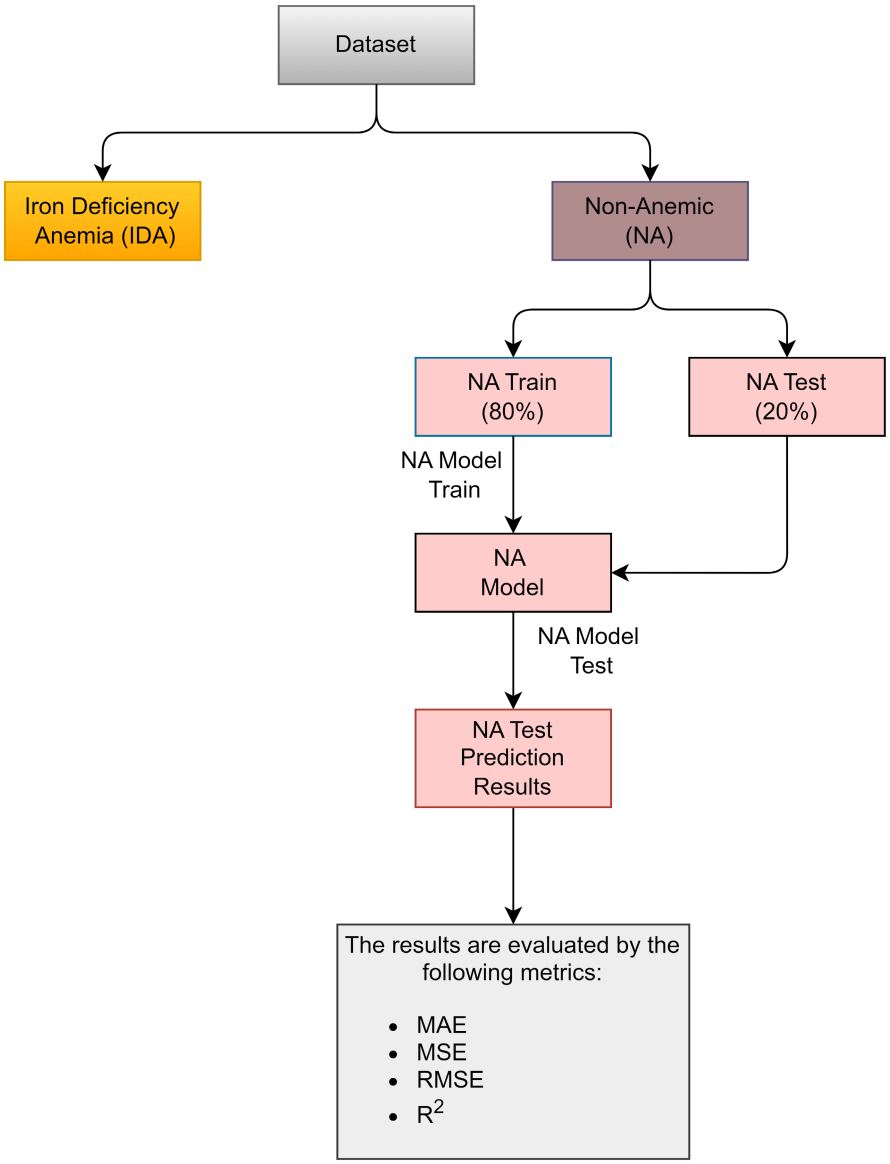

FIGURE 1 | Illustration of the sequential steps involved in implementing HbA1C predictions and conducting evaluations.

the prediction models, the LR method exhibited the highest error value. In general, the ranking of prediction methods' success for all metrics (RFR, SVR, KNR, LR, in order from highest to lowest) is the same. The disparity between the MSE and RMSE results for the best and worst prediction models was 0.092 and 0.106, respectively.

In experimental studies, another result we observe the extent of prediction errors when IDA examples are given to the NA model. Table 3 and Figure 4 present the results of this experiment. When looking at the success of the methods in the experiment, once again, the best result is obtained using the RFR method. In addition, as in other experiments, the order of success of the prediction methods has not changed. When the IDA samples are given to the NA model, the error values increase for all metrics. For instance, in the RFR method, the error value for the MAE metric increased from 0.268 to 0.361 (0.093).

4 | Discussion

HbA1c is an important laboratory test used in the management of diabetes. HbA1c is expressed as a percentage of HbA. Laboratories report reference intervals (RI) or clinical decision limits (CDL) for the interpretation of tests along with patient results. The CDLs for HbA1c in laboratories are reported along with patient results as follows: ≥6.5% (diabetes), 5.7 ≤ HbA1c <6.5% (prediabetes), HbA1c <5.7% (non-diabetic) [31]. However, because HbA1c measures Hb to which glucose is bound, abnormalities in Hb can lead to inaccurate assessment of HbA1c. Accurate diagnosis or monitoring of diabetes cannot be made based on HbA1c levels in such patients. Indeed, in some recent studies, it has been reported that high HbA1c values were obtained in patients with IDA, whereas in other studies, low HbA1c values were observed [32–35]. Considering the potential racial differences in HbA1c levels for a specific glycemic level [36].

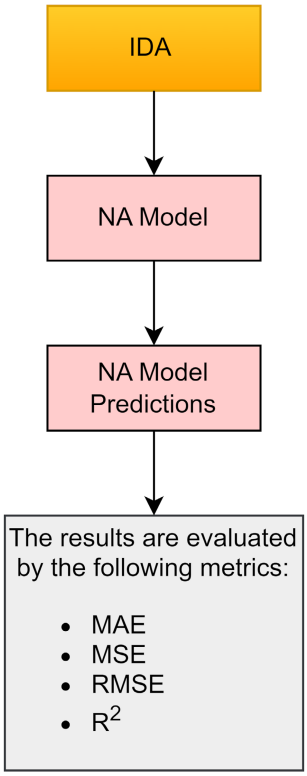

FIGURE 2 | Testing the non-anemic (NA) model with iron deficiency anemia (IDA) samples.

TABLE 2 | Predictions results for the NA model. The model trained and tested with the NA samples.

Model	MAE	MSE	RMSE	R^2
SVR	0.298	0.192	0.438	0.763
LR	0.321	0.235	0.484	0.711
KNR	0.311	0.190	0.436	0.765
RFR	0.268	0.143	0.378	0.824

Abbreviations: KNR, K-nearest neighbor regression; LR, linear regression; MAE, mean absolute error; MSE, mean squared error; NA, non-anemic; RFR, random forest regression; RMSE, root mean squared error; SVR, support vector regression.

TABLE 3 | Predictions results for the NA model. The model trained with the samples with NA and tested with the samples with IDA.

Model	MAE	MSE	RMSE	R^2
SVR	0.375	0.270	0.520	0.735
LR	0.385	0.305	0.553	0.700
KNR	0.391	0.287	0.536	0.718
RFR	0.361	0.242	0.492	0.762

Abbreviations: KNR, K-nearest neighbor regression; LR, linear regression; MAE, mean absolute error; MSE, mean squared error; NA, non-anemic; RFR, random forest regression; RMSE, root mean squared error; SVR, support vector regression.

In our study, we improved the assessment of HbA1c levels in female IDA patients using ML methods for our population. We employed four different prediction methods. We used four

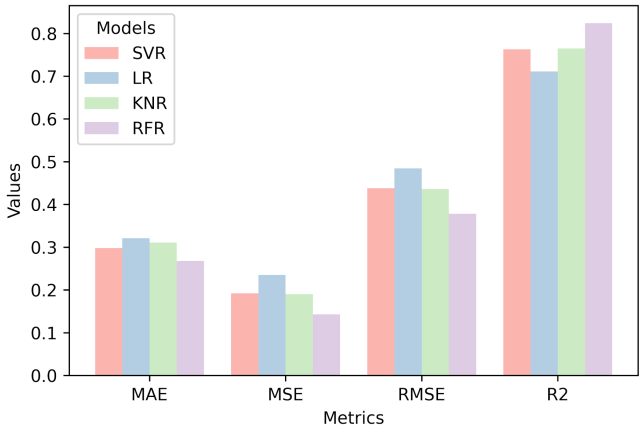

FIGURE 3 | Predictions results for the non-anemic (NA) model.

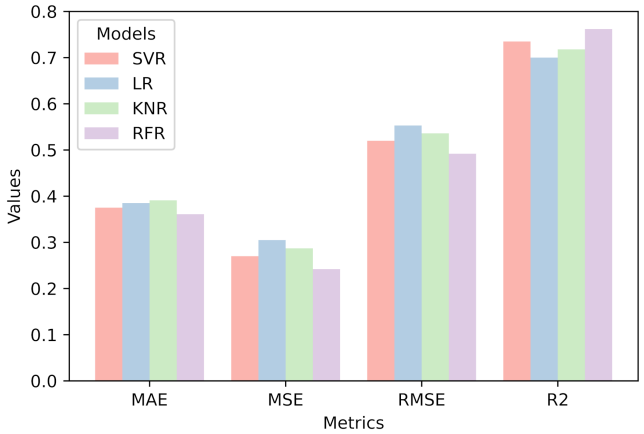

FIGURE 4 | Predictions results for the non-anemic (NA) model. The model was trained with the samples NA and tested with the iron deficiency anemia (IDA) samples.

different evaluation metrics to compare the success of these methods and found that RFR was the most successful method. Therefore, we trained our model according to the RFR method.

Some studies have demonstrated an association between increasing HbA1c concentrations and IDA and a significant decrease in HbA1c following iron therapy. They observed a 0.4% decrease in HbA1c levels accompanying a 2.2 mg/dL increase in hemoglobin after IDA treatment [32, 33]. In contrast to these studies, in a prospective study, it was observed that RIA was associated with low HbA1c levels and that HbA1c levels increased after iron treatment [34]. However, in this study, the research study was terminated while anemia was ongoing and a sufficient and homogeneous number of patients could not be reached to evaluate subgroups.

In another study, it was found that iron treatment did not change HBA1c levels [35]. Blood HbA1c levels increase proportionally with the glucose concentration to which HbA is exposed because erythrocytes constantly facilitate glucose uptake. The excess glucose taken up is irreversibly bound to Hb. The HbA1c test is used to measure the amount of Hb glycated with glucose over the past 2–3 months, reflecting the normal lifespan of RBCs. However, HbA1c levels may rise in some

conditions where RBC lifespan is prolonged because of the effect of prolonged exposure to glucose and irreversible binding [37]. IDA, which prolongs the lifespan of RBCs and exposes the cells to glucose for a longer duration, is associated with the inadvertent elevation of HbA1c levels [36]. In IDA, increased levels of malondialdehyde facilitate the glycation of Hb [34]. In addition, along with a decrease in Hb concentration, the glucose concentration per RBC increase even if serum glucose remains the same. This can increase the glycolytic fraction of Hb [38, 39]. To the best of our knowledgeof the relationship between Hb and HbA1c has been mainly explored in a limited number of small patient groups until now. Our study included a considerably larger number of patients compared with these studies.

Recently, a study conducted in Japan investigated the relationship between Hb and HbA1c levels using machine learning-based large-scale data. The study concluded that the impact of anemia on HbA1c varies according to the population, and the relationship cannot be easily formulated [40]. However, our study, in contrast to this research, focused specifically on predicting HbA1c levels in women with only IDA. The data in this study were obtained from health checkup records and only included the analysis of test results such as Hb, HbA1c, and fasting glucose levels. However, it did not include serum ferritin levels or reticulocyte counts. Our study, on the other hand, was conducted with a more specific group of women, considering parameters that could aid in diagnosing IDA, such as Hb, MCV, MCH, TIBC, UIBC, iron, TS, and ferritin. Therefore, our study is more targeted to a specific group.

In a study involving patients with both Type 2 Diabetes and IDA (956 individuals), iron supplementation was provided for 3 months. When health checkup results before and after iron supplementation were examined in these patients, a U-shaped spline curve was observed between Hb and HbA1c. This curve showed the course of HbA1c levels in individuals with IDA; HbA1c levels increased slightly and decreased during the iron treatment phase. In our study, similar to Takeuchi et al.'s work, we found that we could not definitively state whether HbA1c increased or decreased in IDA patients. Therefore, we considered that our results might be consistent with those of Takeuchi et al. [40]. Ultimately, even though we included patients in our study based on specific criteria for the diagnosis of IDA, some patients might have been taking medications or might have undergone new hemoglobin synthesis.

In our study, ML model training was performed on NA individuals. After training, we evaluated the accuracy of the non-anemic prediction model and the predictions of IDA data using the non-anemic model. We found that the error values increased for all results in predictions made with IDA data. The increase in the found metrics can be informative in determining clinical decisions. When determining these values, the RFR method, which provided the best results among the prediction methods, was considered the basis. Looking at the RFR values in Tables 2 and 3, the MAE value changed to 0.093 (0.361–0.268), the MSE value to 0.099 (0.242–0.143), and the RMSE value to 0.114 (0.492–0.378). Considering these error values, we can conclude that the decision threshold for our dataset could vary around 0.1. In our study, we found that some clinical decision changes occurred in some IDA patients. According to our results, a 0.1 unit change in HbA1C values resulted in a clinical decision change in 49 (4%) out of 1116 nondiabetic individuals, 34 (9%) out of 346 prediabetic individuals, and 15 (3%) out of 406 diabetic individuals.

In a recent retrospective study evaluating approximately 12,000 IDA and 21,000 non-IDA patients based on laboratory data, it was showed that HbA1c concentrations in IDA patients may be higher. Researchers emphasized the importance of clinicians considering the IDA condition before making therapeutic decisions based on HbA1c levels [17]. Therefore, the results of our study will be beneficial in clinical practice. We considered implementing the RFR method, which we found to be the most effective ML method in our study, in our laboratory. Thus, we could report the HbA1c results generated for our IDA patients along with device measurement results. During reporting, providing an additional result for HbA1c obtained with ML and stating that it is derived from ML could guide the physician in evaluating the patient.

In addition to the CDLs established for HbA1c, recent guidelines have emphasized an individualized approach in monitoring patients diagnosed with diabetes. It is recommended to apply different HbA1c target ranges based on the patient's clinical condition, comorbidities, prescribed medication treatments, and life expectancy [36]. In this context, the application of different individual CDLs for HbA1c in the monitoring of diabetic patients comes to the forefront. As a result, reporting the HbA1c results of patients with IDA, along with the results obtained using the RFR method, will be beneficial in the management of the disease.

This study is essentially an application of the method of seeking answers to the question "How can we present an analysis result more accurately to clinicians?" that we have conducted in our laboratory. As laboratory professionals, our aim is always to provide the most reliable results and to improve the quality of the laboratory results. Here, the sample is affected in vivo during the pre-analytical process before it reaches the laboratory. There is a situation in which one disease affects the test of another disease. It is likely that the test, which is used as a definitive diagnostic criterion for diabetes, is affected by a pre-pre-analytical error caused by the prolonged lifespan of erythrocytes and their prolonged exposure to blood glucose in another disease. Pre-analytical errors account for approximately 60%–70% of the sources of errors in laboratories, and they are among the most difficult errors to prevent. In particular, preventing the impact of the test being analyzed due to a situation that occurs in vivo is very difficult. In this study, we attempted to prevent this pre-analytical error and observed changes in clinical decision-making. Therefore, our study will also make valuable contributions to the fields of laboratory and endocrinology.

5 | Limitations and Future Work

In our study, we obtained only one result for each patient. Therefore, at the time of obtaining the results, we did not have information about the patient's medication use or how long they had been diagnosed with IDA. Consequently, we could not differentiate between patients who received iron supplementation

due to IDA and those who did not. Additionally, not knowing whether there were patients with hemoglobin variants in our study group could be a limitation of the study. Because our study reflects our own population, we believe that demonstrating its applicability in other laboratories is necessary.

6 | Conclusion

It is remarkable to see that the results obtained with machine learning can contribute to a more accurate assessment of HbA1c levels, especially in women with IDA, and may lead to decision changes, particularly in cases where HbA1c levels are near medical decision limits. Our study is expected to contribute to a more accurate evaluation of HbA1c levels in women with IDA. Using machine learning to analyze HbA1c results in women with IDA may unveil distinctions among patients whose HbA1c values hover near critical medical decision thresholds. This intersection of technology and laboratory science holds promise for enhancing precision in medical decision-making processes.

Author Contributions

This study was produced from a thesis project by Fatma Ceyla Eraldemir and Irfan Kösesoy, who share senior authorship. Kadra Mohamed Abdillahi, Fatma Ceyla Eraldemir, and Irfan Kösesoy contributed equally to this work and share the first authorship. All authors have accepted responsibility for the entire content of this manuscript and have approved its submission.

Ethics Statement

Ethics approval was provided by KOU GOEK.

Consent

All authors have consented to the publication of this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available on request from the corresponding author.

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