

## HbA1c: A REVIEW OF NON-GLYCAEMIC VARIABLES

Novita Retika\*

*\*Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia*

**\*Corresponding Author:**

[novitaretika@gmail.com](mailto:novitaretika@gmail.com)

---

### Abstract

**Introduction:** People at risk for diabetes and those with high HbA1c are monitored for sugar levels. Age, race, gender, erythrocyte turnover, anemia, pregnancy, Haemoglobin variations, thyroid, liver, HIV, and renal disorders impact HbA1c nonthermally. Different forms of hemoglobin have long been recognized to alter HbA1c production and measurement. This interference depends on the congenital disease that affects hemoglobin production and the HbA1c measurement method.

**The aim:** This article showed non-glycaemic variables in HbA1c examination.

**Methods:** By comparing itself to the standards set by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020, this study was able to show that it met all of the requirements. So, the experts were able to make sure that the study was as up-to-date as it was possible to be. For this search approach, publications that came out between 2013 and 2023 were taken into account. Several different online reference sources, like Pubmed and SagePub, were used to do this. It was decided not to take into account review pieces, works that had already been published, or works that were only half done.

**Result:** In the PubMed database, the results of our search brought up 102 articles, whereas the results of our search on SagePub brought up 87 articles. The results of the search conducted for the last year of 2013 yielded a total 34 articles for PubMed and 21 articles for SagePub. In the end, we compiled a total of 19 papers, 11 of which came from PubMed and eight of which came from SagePub. We included seven study that met the criteria.

**Conclusion:** HbA1c is straightforward, accurate, and on-site. It can diagnose and predict diabetes in low- and middle-income countries and remote areas. Hyperglycemia—diabetes' biochemical hallmark—should be properly controlled to reduce complications. Age, ethnicity, gender, erythrocyte turnover, anemia, pregnancy, haemoglobin variations, thyroid, liver, HIV, and renal diseases affect HbA1c measurement.

**Keyword:** Demographic; HbA1c examination; Inflammation; Non-glycaemic variables; Pregnancy

**INTRODUCTION**

Diabetes mellitus encompasses a cluster of metabolic disorders characterized by hyperglycemia, initially due to insulin resistance, impaired insulin secretion, insulin deficiency, increased glucose production, and decreased glucose utilization.<sup>1</sup> The resulting complications associated with this condition constitute a significant global cause of mortality. The metabolic process of carbohydrates is hindered in the cells of the body due to a deficiency or absence of insulin, leading to the body resorting to the breakdown of its own protein, fat, and glycogen. This metabolic imbalance ultimately results in the condition known as hyperglycemia.<sup>2,3</sup>

Rahbar et al. found HbA1c 1969. A tetramer of alpha and beta globin chains is Hb. High blood glucose non-enzymatically glyicates hemoglobin at numerous sites. Protein amino groups receive sugar residue from non-enzymatic glycolation.<sup>4</sup> HbA1c, or glycated Hb, measures a patient's glycemic state for the past 3 months or 8 to 12 weeks and offers glucose control parameters. Recent exercise and eating do not influence it. The recommended diabetes diagnosis cutoff is 6.5% HbA1c. Human Adult Hb consists of HbA (97% of the total, it is made up of 4 polypeptide chains, 2 $\alpha$  and 2 $\beta$ ), HbA2 (2.5% , made up of 2 $\alpha$  and 2 $\delta$ ) and HbF (0.5%, and made up of 2 $\alpha$  and 2 $\gamma$ ).<sup>5-7</sup>

Chromatographic study of HbA1 shows that it has a few minor hemoglobins. These are HbA1a, HbA1b, and HbA1c, which are called fast hemoglobins, glycohemoglobins, or glycated hemoglobins.<sup>4</sup> HbA1c is made when the aldehyde group of glucose and hexoses binds to the amino terminal of the  $\beta$ -chain of Hb, forming an unstable Schiff base called aldimine (pre-HbA1c) that then goes through an Amadori rearrangement to become a more stable ketoamine called HbA1c.<sup>7</sup> This process, called glycation, takes 120 days. So, this property of Hb is used to track the average amount of glucose in the blood.<sup>5,8</sup>

So, HbA1c is used to check how well blood sugar is controlled in people who are at risk for diabetes and to keep an eye on sugar levels in people whose HbA1c is high. Non-glycemic factors that affect HbA1c are age, race, gender, erythrocyte turnover, anemia, pregnancy, Haemoglobin variants, thyroid disease, liver disease, HIV, and kidney diseases. It has been known for a long time that different types of hemoglobin can affect the production and measurement of HbA1c. This interference depends on the type of congenital disorder that affects hemoglobin synthesis and the method used to measure HbA1c.<sup>8-11</sup>

The present investigation revealed the presence of non-glycemic factors in the assessment of HbA1c.

**METHODS**

The investigator of this investigation employed measures to guarantee full compliance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines. The purpose of implementing this strategy is to ensure the precision of the investigation's findings. The primary aim of this review was to present non-glycaemic factors that influence the measurement of HbA1c. The main aim of this study is to underscore the significance of the aforementioned subjects discussed in the book.

In order to qualify for inclusion in the study, researchers were required to satisfy specific criteria. These criteria included the prerequisite that the paper be written in the English language and that it primarily address non-glycaemic variables in the assessment of HbA1c. In order for the paper to be published, it is imperative that it fulfills both of these stipulations. A number of the publications being evaluated were published within the period of 2013 and the predetermined timeframe considered relevant for the objectives of this systematic review. Prohibited in the academic context are editorials, submissions without a Digital Object Identifier (DOI), review articles that have been previously published, and submissions that effectively duplicate previously published journal papers.

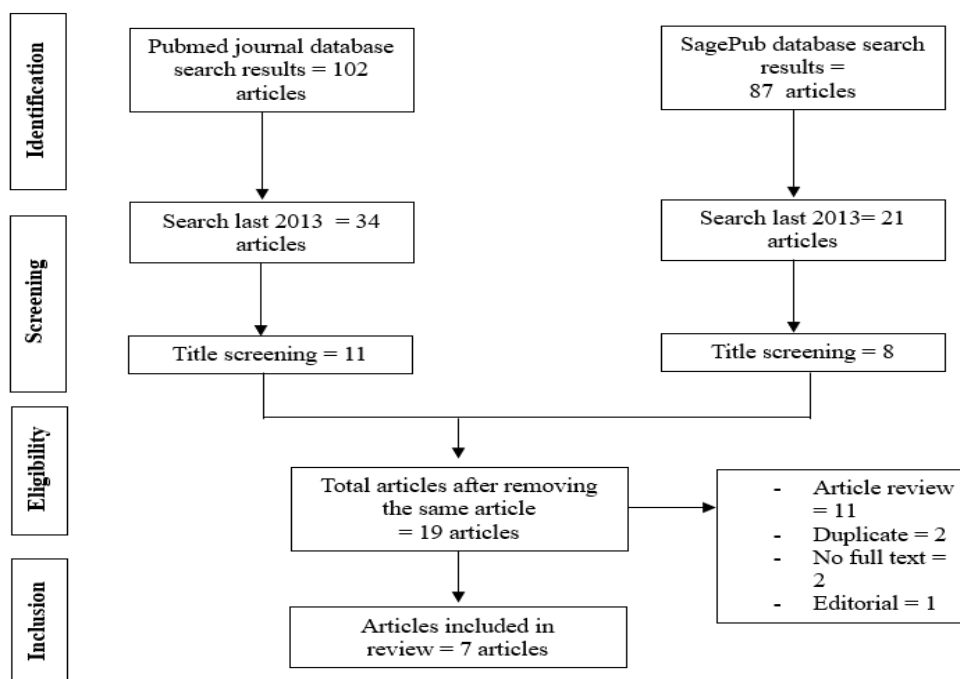


Figure 1. Article search flowchart

We used “non-glycaemic variables” and “HbA1c” as keywords. The search for studies to be included in the systematic review was carried out from August, 27<sup>th</sup> 2023 using the PubMed and SagePub databases by inputting the words: (*"non-glycaemic"[All Fields] AND ("variabilities"[All Fields] OR "variability"[All Fields] OR "variable"[All Fields] OR "variable s"[All Fields] OR "variables"[All Fields] OR "variably"[All Fields]) AND ("glycated hemoglobin"[MeSH Terms] OR ("glycated"[All Fields] AND "hemoglobin"[All Fields]) OR "glycated hemoglobin"[All Fields] OR "hba1c"[All Fields] OR "hba1cs"[All Fields])) AND ((y\_10[Filter]) AND (clinicaltrial[Filter]))*) used in searching the literature.

The researchers assessed the abstract and title of each paper in order to ascertain its adherence to the inclusion criteria. The writers subsequently identified and selected relevant research from the existing literature to be utilized as sources for the essay. In order to arrive at this result, an extensive analysis was conducted on multiple studies that exhibited a consistent pattern. Prior to submission, it is imperative that all written works are composed in the English language and have not been previously published. The systematic review exclusively included publications that satisfied all of the predetermined inclusion criteria. This restricts the search outcomes to solely those that are relevant to the user's query. The outcomes of studies that fail to meet our established standards are disregarded. The research findings will thereafter undergo a comprehensive analysis.

This research's investigation yielded the following information: names, authors, publication dates, location, study activities, and parameters. Before deciding which publications to investigate further, each author performed independent research on the research included in the publication's title and abstract. The subsequent step is to evaluate all of the articles that satisfy the inclusion criteria for the review. Then, we will choose which articles to include in the review based on the findings. This criterion is used to select documents for additional evaluation. To facilitate as much as possible the selection of papers for evaluation. This section discusses the prior studies conducted and the factors that led to their inclusion in the review.

## RESULT

In the PubMed database, the results of our search brought up 102 articles, whereas the results of our search on SagePub brought up 87 articles. The results of the search conducted for the last year of 2013 yielded a total 34 articles for PubMed and 21 articles for SagePub. In the end, we compiled a total of 19 papers, 11 of which came from PubMed and eight of which came from SagePub. We included seven study that met the criteria.

Chen, et al (2023)<sup>12</sup> found a significant inverse association between dietary Mg intake and HGI ( $\beta = -0.00016$ , 95%CI: -0.0003, -0.00003,  $P = 0.019$ ). Dose-response analyses revealed that HGI decreased with increasing intakes of Mg when reached the point above 412 mg/day. There was a linear dose-response relationship between dietary Mg intake and HGI in diabetic subjects, and there was an L-shape dose-response relationship in non-diabetic individuals. Increasing the intake of Mg might help lower the risk associated with high HGI. Further prospective studies are requested before dietary recommendations.

Hempe, et al (2021)<sup>13</sup> showed significant correlation between a greater Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and those of black race, regardless of their diabetes status. Additionally, older age, higher body mass index (BMI), and elevated C-reactive protein (CRP) levels were also found to be linked with a higher HGI in individuals without diabetes and those with prediabetes, but not in those with diabetes. In the population of self-reported diabetes people, the average Hemoglobin Glycation Index (HGI) was observed to be 0.6% higher. There was no observed association between the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), 2-hour Oral Glucose Tolerance Test (OGTT), or plasma insulin levels and the Homeostatic Glucose Infusion (HGI) in individuals classified as normal, prediabetic, or diabetic.

Kalairajan, et al (2019)<sup>9</sup> showed the average baseline HbA1c level in individuals with anemia (4.62%) was found to be considerably lower compared to the control group (5.45%,  $p < 0.001$ ). A notable rise was seen in the patients' HbA1c values three months post-treatment (5.82%,  $p < 0.001$ ). A notable link was identified between the levels of haemoglobin and HbA1c in the study group prior to correction (correlation coefficient [CC] = 0.26,  $p < 0.01$ ). This study demonstrates a correlation between the treatment of iron-deficient anemia and an increase in HbA1c levels.

Benaiges, et al (2017)<sup>14</sup> showed rule-out threshold for HbA1c of 4.8% (29 mmol/mol) had 96.7% sensitivity (95%CI = 93.9–99.5), 10.1% specificity (95%CI 8.3–12.0) and a negative predictive value of 95.3% (95%CI = 91.3–99.3). A rule-in value of 5.6% (38 mmol/mol) had a positive predictive value of 31.6% (95%CI = 24.4–38.9), 89.3% specificity (95%CI = 87.4–91.2) and 32.9% sensitivity (95%CI = 25.4–40.4). The low positive predictive value of the rule-in threshold precludes its use for GDM diagnosis, but could be used to identify women at high risk of GDM in whom the diagnosis can be established using a one-step approach. The overall saving of the proposed algorithm would be 6.5% of the total cost with the standard strategy.

**Table 1. The literature include in this study**

Author	Origin	Method	Sample Size	Factor influence	Result
<b>Chen, 2023<sup>12</sup></b>	China	Prospective cohort study	89 patients	Dietary magnesium (Mg) on hemoglobin glycation index (HGI)	Augmenting the consumption of magnesium (Mg) may potentially mitigate the risk linked to elevated glycemic index (HGI). Additional prospective research are required prior to formulating dietary recommendations.
<b>Hempe, 2021<sup>13</sup></b>	United State of America	Prospective cohort study	18,675 diabetes treatment-naïve adults without self-reported diabetes	Race, age, BMI and CRP	The regression equation obtained from the diabetes treatment-naïve adult NHANES reference population, which consists of individuals from diverse demographic backgrounds, can be effectively utilized to standardize the calculation of the HGI. This standardization is applicable in both clinical practice and research settings, as it allows for a mechanistic understanding of the population-level variation in the HGI and the underlying reasons why a higher HGI is linked to an increased risk of chronic vascular disease.
<b>Kalairajan, 2019<sup>9</sup></b>	United State of America	Cross sectional	120 patients confirmed	Iron deficiency anaemia treatment	The findings of this study indicate a positive correlation between the therapy of iron deficient anaemia and a rise in HbA1c levels.
<b>Benaiges, 2017<sup>14</sup></b>	Spain	Prospective cohort study	1,195 pregnancy woman	Pregnancy	The sensitivity and specificity of a first-trimester HbA1c test are not enough for the diagnosis of gestational diabetes mellitus (GDM). However, employing different threshold values for the test could potentially streamline the diagnostic procedure by decreasing the need for oral glucose tolerance tests, lowering associated expenses, and minimizing annoyance for patients.
<b>Liu, 2015<sup>15</sup></b>	United State of America	Cross sectional	7,323 nondiabetic participants	Inflammation, race, BMI	The study examines the relationship between HGI and the impact of inflammation on HbA1c levels in a sample of U.S. people without diabetes. The findings suggest that HGI could serve as an indicator of inflammation-related risk factors, regardless of factors such as fasting plasma glucose levels, race, and obesity.
<b>Renz, 2015<sup>16</sup></b>	Brazil	Cross sectional	262 women in the third trimester of gestation	Pregnancy	The findings of this study indicate that the integration of HbA1c and OGTT measures could potentially serve as a valuable diagnostic tool for GDM.
<b>Shipman, 2015<sup>17</sup></b>	United Kingdom	Cross sectional	Nine hundred and forty-eight patients consisting of 711 white subjects (407 women) and 237 South Asian subjects (138 women) were studied	Racial	The elevated occurrence of hematological irregularities among individuals of South Asian descent, along with their higher adjusted levels of HbA1c and fructosamine but lower levels of fasting glucose in comparison to individuals of white ethnicity, indicate that ethnic disparities in glycation markers may be attributed, at least partially, to a combination of erythrocyte-related factors and glycation processes independent of glycemia.

Liu, et al (2015)<sup>15</sup> showed mean HbA1c, CRP, monocyte, and PMNL levels, but not FPG, progressively increased in the low, moderate, and high HGI subgroups. There were disproportionately more Blacks than whites in the high HGI subgroup. CRP ( $\beta = 0.009$ ; 95% confidence interval [CI] = 0.0001-0.017), PMNL ( $\beta = 0.036$ ; 95% CI = 0.010-0.062), and

monocyte count ( $\beta = 0.072$ ; 95% CI = 0.041-0.104) were each independent predictors of HGI after adjustment for age, sex, race, triglycerides, hemoglobin level, mean corpuscular volume, red cell distribution width, and obesity status.

Renz, et al (2015)<sup>16</sup> conducted a study with 262 women in the third trimester. HbA1c  $\geq 40$  mmol/mol (5.8%) showed adequate specificity in diagnosing GDM (94.9%) but low sensitivity (26.4%). Unlike, HbA1c values of 31 mmol/mol (5.0%) presented adequate sensitivity (89.7%) but low specificity (32.6%) to detect GDM. For women with HbA1c  $\geq 40$  mmol/mol (5.8%), the positive and negative likelihood ratios were 5.14 (95%CI = 2.49–10.63) and 0.78 (0.68–0.88), respectively. The post-test probability of GDM was about 40%, representing a 4.0-fold increase in the mean pre-test probability. This cut-off point could eliminate the need for the unpleasant and laborious OGTT tests in almost one third of cases, as 38% of patients with GDM may be diagnosable by HbA1c test alone.

Shipman, et al (2015)<sup>17</sup> showed that South Asians had higher HbA1c concentrations (5.9 % vs. 5.8 % mmol/mol,  $p = 0.011$ ) than whites. South Asians also had lower hemoglobin, ferritin and vitamin B12 concentrations than whites. After adjustment for independent variables, South Asian ethnicity was associated with higher HbA1c concentrations (0.89, 95 % CI = 0.06–1.72,  $p = 0.035$ ), higher fructosamine levels (3.93, 95 % CI = 0.79, 7.08,  $p = 0.014$ ) and lower fasting plasma glucose concentrations ( $-0.12$ , 95 % CI =  $-0.26$  to  $-0.02$ ,  $p = 0.026$ ) compared to white race.

## DISCUSSION

Over the past two decades, type 1 and type 2 DM has increased globally and is predicted to continue. Several chronic consequences of diabetes increase morbidity and mortality. An accurate, easy-to-manage HbA1c test with onsite results. It can diagnose and prognose diabetes in low- and middle-income nations and hard-to-reach people. Both the level of and exposure to hyperglycemia, as well as glycemic variability, contribute to the pathogenesis of diabetic complications, with different patterns of disease pathogenesis in patients with type 1 or type 2 diabetes. Despite its analytical and biological limitations, HbA1c remains the key biomarker of long-term glycemic control.<sup>18,19</sup>

Various non-glycemic factors that have an impact on the measurement of HbA1c include age, ethnicity, gender, erythrocyte turnover, anaemia, pregnancy, haemoglobin variations, thyroid illness, liver disease, HIV, and kidney diseases. The interference of hemoglobin variations on HbA1c production and measurement has been well acknowledged, with the extent of this interference being contingent upon the specific congenital condition affecting hemoglobin synthesis as well as the analytical methodology employed for HbA1c measurement.<sup>6,11,13,14,16</sup>

The study revealed a correlation between age and a progressive rise in HbA1c levels among individuals without diabetes, regardless of gender and glycemic status.<sup>13</sup> This suggests that establishing age-specific reference intervals or clinical cut-off points could enhance the diagnostic and therapeutic precision of the HbA1c test for diabetes. Ethnic disparities in HbA1c values persist despite equivalent glycemia levels. A recent meta-analysis has demonstrated that individuals of Caucasian descent have marginally lower HbA1c values compared to individuals from different ethnic backgrounds.<sup>11,20</sup> Increasing the intake of Mg and iron might help lower the risk associated with high HGI.<sup>9,12</sup>

Various non-glycemic factors have been identified as influential in the levels of HbA1c, such as erythropoiesis, hemoglobin production, and circumstances that impact the lifespan of red blood cells. Deficiency anemias typically result in elevated HbA1c levels due to the presence of old erythrocytes, which are more prevalent in individuals with this condition. Conversely, hemolytic anemias of any etiology may lead to lower HbA1c levels. Nonhematological conditions influencing HbA1c values include pregnancy, chronic renal failure and certain medications.<sup>14,18,20</sup>

## CONCLUSION

HbA1c is accurate, simple, and available onsite. It can help diagnose and prognose diabetes in low- and middle-income nations and hard-to-reach populations. Diabetes' biochemical hallmark, hyperglycemia, should be tightly controlled to reduce the risk of complications. Non-glycemic variables that affect HbA1c measurement include age, ethnicity, gender, erythrocyte turnover, anemia, pregnancy, haemoglobin variations, thyroid disease, liver disease, HIV, and renal disease.

## REFERENCES

- [1]. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, et al. 2019 update to: Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*. Februari 2020;63(2):221–8.
- [2]. Doshi SM, Friedman AN. Diagnosis and Management of Type 2 Diabetic Kidney Disease. *Clin J Am Soc Nephrol*. Agustus 2017;12(8):1366–73.
- [3]. Swarup S, Goyal A, Grigorova Y, Zeltser R. Metabolic Syndrome. *StatPearls*. Mei 2022;
- [4]. Gupta S, Jain U, Chauhan N. Laboratory diagnosis of HbA1c: a review. *J Nanomed Res*. 2017;5(4):120.
- [5]. Bloomgarden Z. Beyond HbA1c. *J Diabetes*. 2017;9(12):1052–3.
- [6]. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights*. 2016;11:11:BMI-S38440.
- [7]. Selvin E. Are there clinical implications of racial differences in HbA1c? A difference, to be a difference, must make a difference. *Diabetes Care*. 2016;39(8):1462–7.
- [8]. English E, Lenters-Westra E. HbA1c method performance: the great success story of global standardization. *Crit*

- Rev Clin Lab Sci. 2018;55(6):408–19.
- [9]. Kalairajan S, AR M. A study on influence of iron deficiency anaemia over HbA1c levels. 2019;
- [10]. Chehregosha H, Khamseh ME, Malek M, Hosseinpanah F, Ismail-Beigi F. A view beyond HbA1c: role of continuous glucose monitoring. *Diabetes Ther.* 2019;10:853–63.
- [11]. Forbes A, Murrells T, Mulnier H, Sinclair AJ. Mean HbA1c, HbA1c variability, and mortality in people with diabetes aged 70 years and older: a retrospective cohort study. *lancet Diabetes Endocrinol.* 2018;6(6):476–86.
- [12]. Chen J, Lin S, Wang X, Wang X, Gao P. Lower Dietary Magnesium Is Associated with a Higher Hemoglobin Glycation Index in the National Health and Nutrition Examination Survey. *Biol Trace Elem Res.* Juni 2023;
- [13]. Hempe JM, Yang S, Liu S, Hsia DS. Standardizing the haemoglobin glycation index. *Endocrinol diabetes Metab.* Oktober 2021;4(4):e00299.
- [14]. Benaiges D, Flores-Le Roux JA, Marcelo I, Mane L, Rodríguez M, Navarro X, et al. Is first-trimester HbA1c useful in the diagnosis of gestational diabetes? *Diabetes Res Clin Pract.* 2017;133:85–91.
- [15]. Liu S, Hempe JM, McCarter RJ, Li S, Fonseca VA. Association between Inflammation and Biological Variation in Hemoglobin A1c in U.S. Nondiabetic Adults. *J Clin Endocrinol Metab.* Juni 2015;100(6):2364–71.
- [16]. Renz PB, Cavagnoli G, Weinert LS, Silveiro SP, Camargo JL. HbA1c test as a tool in the diagnosis of gestational diabetes mellitus. *PLoS One.* 2015;10(8):e0135989.
- [17]. Shipman KE, Jawad M, Sullivan KM, Ford C, Gama R. Ethnic/racial determinants of glycaemic markers in a UK sample. *Acta Diabetol.* 2015;52:687–92.
- [18]. Campbell L, Pepper T, Shipman K. HbA1c: a review of non-glycaemic variables. *J Clin Pathol.* 2019;72(1):12–9.
- [19]. Kashima S, Inoue K, Matsumoto M, Akimoto K. Do non-glycaemic markers add value to plasma glucose and hemoglobin a1c in predicting diabetes? Yuport health checkup center study. *PLoS One.* 2013;8(6):e66899.
- [20]. Schnell O, Crocker JB, Weng J. Impact of HbA1c testing at point of care on diabetes management. *J Diabetes Sci Technol.* 2017;11(3):611–7.