Original Research Article

Influence of age, sex and body mass index on the levels of glycosylated haemoglobin among non-diabetic Nigerian population

ABSTRACT

The influence of age and sex on the levels of glycosylated haemoglobin among non-diabetic Nigerian population were investigated in this study. Seventy-nine non-diabetic individuals volunteered for the study and were grouped into male and female and then into four groups according to age: ≤ 20 years, 21 - 40 years, 41 - 60 years and ≥ 61 years. Fasting blood glucose, 2-hour post-load glucose, packed cell volume and genotype analyses of subjects were initially determined to ensure that subjects were non-diabetic and had no glucose metabolic impairment. Subsequently, glycosylated haemoglobin and body mass index were measured. Student's t-test, Pearson correlation and one-way analysis of variance were used to compare the data which were presented as a mean ± standard deviation. Statistical significance was accepted at $p < 0.05$. The results obtained showed that: (1) glycosylated haemoglobin (HbA1c) significantly increased with age, (2) there is no correlation between HbA1c with sex and (3) there was a positive association between Hba1c and body mass index in normal glucose tolerant subjects. Based on the result of this study, the contributions of age and BMI to HbA1c levels should be taking into account when making diagnostic and therapeutic decisions with regard to diabetes care using HbA1c.The hba1c range of (4.0 - 5.2) % could be considered as the normal range for individuals below sixty-one years while the HbA1c level of ≤ 5.27% is suggested for individuals above sixty years. However, further studies are required especially to investigate the non-glycaemic factors affecting HbA1c levels in normal glucose tolerant populations so as to really understand the actual role glycosylated haemoglobin values play in diabetes management and diagnosis.

Keywords: Age, sex, glycosylated haemoglobin, non-diabetic, body mass index

1. Introduction

Studies on chronic complications of diabetes established the role of glycosylated haemoglobin, HbA1c, as a marker of evaluation of long-term glycaemic control, glycaemic risk and prediction of diabetic complications, and as a screening tool for the diagnosis of diabetes [1,2]. It is considered as one of the best achievements in the history of diabetes mellitus. HbA1c is a specific haemoglobin produced by a two-stage non-enzymatic attachment of glucose molecule to the N-terminal valine of the ß-chains of the haemoglobin molecule. Once formed, the HbA1c remains throughout the life span of the erythrocyte. Hence, it is primarily measured to identify the average plasma glucose concentration over the previous 2-3months. If other factors that may affect the HbA1c levels such as haemoglobinopathies, anaemia, etc, are kept constant, normal levels of plasma glucose will produce a normal amount of HbA1c. Hence, HbA1c level will increase in a predictable way and so serves as a marker of glycaemic control. HbA1c measurement is the most preferred test by clinicians and patients for monitoring glycaemia. This is because, its measurement has substantially less biologic variability, needing no fasting or timed samples and it is a better index of overall glycaemic exposure and risk for long-term complications [3]. However, certain studies have suggested that factors such as age, sex and body mass index

may affect HbA1c levels and hence, its use as a marker of glycaemic control and as well as its usefulness in diabetes diagnosis and management. According to the available research reports in this regard, there seems to be no agreement on whether age and sex have a significant effect on HbA1c values. While some studies suggest a positive association between HbA1c levels with age and sex, [4-6], others indicated no association of HbA1c levels with neither age nor sex, [7-9]. The present study is aimed at investigating the influence of age, gender and body mass index on the levels of HbA1c among non-diabetic Nigerian population.

2. Materials and Methods

2.1 Study Population

A total of seventy-nine healthy non-diabetic Nigerian males and females of age ranging from eleven (11) to (70) years participated in the study. The subjects were a mixture of lecturers, civil servants, farmers and students with varying levels of socioeconomic status. They were apparently healthy individuals, with no identifiable disease and were not on any medications known to affect glucose metabolism. Pregnant women and individuals, who had received treatment for anaemia, received or donated blood within the last one month prior to the study, were excluded from the study.

2.2 Ethical Clearance

Considering the nature of this research involving human volunteers, ethical clearance (with certificate number: NHREC/05/01/2008B-FWA00002458-1RB00002323) was sought and obtained from the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State. Informed consent was also obtained prior to the study, from all participating subjects. For minors, that is those subjects below the age of eighteen years of age, consent was obtained from their parents or guardians.

2.3 Experimental Design

Glucose tolerance test (comprising of fasting blood sugar and 2-hour post-load glucose tests), packed cell volume and haemoglobin genotype determination were performed as a screening criterion for participation in the study. The subjects were grouped according to sex. Each of the sex group was subdivided into four subgroups according to age-ranges (in years):

AgeGroups (in years)	*Sex*	
	Men	Women
≤ 20		
21-40		
41 – 60		
≥ 61		

2.4 Anthropometric Data Collection

The body weight of subjects was measured in kg with a minimal amount of clothing using Hana Bathroom Scale while their heights were measured to the nearest 0.1cm with the subject standing erect, barefooted and without scarf or cap against a wall using a calibrated ruler. The body mass index (BMI) was calculated as the ratio of body weight in kg to the height in square meters.

2.5 Sample Collection

Whole blood was used in all the analysis. Blood for fasting blood glucose measurement was collected from subjects by finger-prick after an overnight fast (8-12hours). While blood for 2-hour post-load glucose measurement was collected from subjects by finger-prick after 2 hours of the high glycaemic meal (Lucozade boost) containing 75g glucose for all adults and adjusted for weights in children. About 5 millimetres of venous

blood was collected from each subject and transferred into appropriately labelled EDTA bottle for glycosylated haemoglobin, packed cell volume, and genotype analysis.

2.6 Biochemical Analysis

Fasting blood glucose and 2-hour post-load glucose were determined based on the glucose oxidase method as described by Trinder, [10]. Determination of glycosylated haemoglobin (HbA1c) was carried out using an ion-exchange kit (VitroScient) designed based on the method described by Trivelli*etal*. [11]. Packed cell volume was determined by the centrifugation method as described by the National Committee for Clinical Laboratory Standards [12]. Haemoglobingenotypedeterminationwas by the electrophoresis method as described by Schneider [13].

2.7 Statistical Analysis

Student's t-test, Pearson correlation and One-way analysis of variance, (ANOVA) were used to compare the data using statistical package for social sciences (SPSS) version 18. The results were presented as a mean ± standard deviation for continuous variables. The means were separated using Duncan multiple tests. Statistical significance was accepted at $p<0.05$.

3. Results

3.1 Levels of Glycosylated Haemoglobin (HbA1c) According to Age

Table 1 shows the mean HbA1c levels according to age only. Results obtained show that the mean levels of HbA1c increased across the age groups. The increases were significant ($p<0.05$) compared to the age group "≤ 20" year-old as the baseline.

Table 1: Mean HbA1c of Subjects Based on Age

Age groups (years)	HbA1c (%)
≤ 20	4.27 ± 0.64 (22)
21-40	4.97 ± 0.61* (24)
41-60	5.13 ± 0.71* (23)
Above 60	5.26 ± 0.49* (10)

Values represent mean ± standard deviation.
*. Means are statistically significant at $p< 0.05$
(n = 79)
n = number of subjects.
Numbers in parentheses indicate a number of subjects in different age groups.

3.2 Levels of Glycosylated Haemoglobin (HbA1c) According to Sex

Table 2 shows the mean HbA1c levels according to sex at different age groups. There was a sequential increase along the age groups in both men and women. The increases observed were significant ($p<0.05$) when compared to the baseline ("≤ 20" year-old age group) within each gender but not across sex. The highest elevation of HbA1c occurred between the age groups "≤ 20" year-old and "21 to 40" year-old, in both sex, with differences of 0.67 % and 0.76 % respectively. Men had higher mean HbA1c levels than women across the age groups.

Table 2: Mean Glycosylated Haemoglobin of Subjects Based on Sex

Age groups (years)	Men (n = 40) HbA1c (%)	Women (n = 39) HbA1c (%)

≤ 20	4.42 ± 0.72 (12)	4.08 ± 0.51 (10)
21-40	5.09 ± 0.57[a] (12)	4.84 ± 0.64[b] (12)
41-60	5.18 ± 0.64[a] (11)	5.09 ± 0.79[b] (12)
Above 60	5.27 ± 0.67 [a] (5)	5.25 ± 0.30[b] (5)

Values represent mean ± standard deviation.
[a] means are statistically significant at $p < 0.05$ within the men sex.
[b] means are statistically significant at $p < 0.05$ within the women sex.
n = number of subjects.
Numbers in parentheses indicate a number of subjects in different age groups.

3.3 Mean Body Mass Index of Subjects Based on Age

Table 3 shows the mean values for body mass index of the subjects based on age only. The average body mass index showed an increasing trend up to the age group "41 to 60" year-old and then declined slightly. These values significantly ($p<0.05$) increased along the age groups when the age group "≤ 20" year-old was used as the baseline.

Table 3: Mean Body Mass Index of Subjects Based on Age

Age Groups (years)	BMI [Kg/m^2] (n = 79)
≤ 20	19.4 ± 4.1*(22)
21-40	24.2 ± 3.8* (24)
41-60	26.6 ± 4.1* (23)
Above 60	26.0 ± 2.7* (10)

Values represent mean ± standard deviation.
*. Means are statistically significant at $p< 0.05$
n = number of subjects. Numbers in parentheses indicate a number of subjects in different age groups.

4. Discussion

This study investigated the influence of age and sex on the levels of glycosylated haemoglobin (HbA1c) among non-diabetic Nigerian population. Glucose tolerance tests (i.e. fasting blood sugar and 2-hour post-load glucose tests), genotype analyses, packed cell volume, body mass index and HbA1c measurements, and a questionnaire on medical history and lifestyle were carried out on the subjects. Apart from the body mass index and HbA1c measurements, the questionnaire and the other tests were done to ensure that the subjects had no identifiable diseases and were not on any medication known to affect glucose metabolism. Subjects were grouped into men and women, and then into four groups according to age groups: ≤ 20 years, 21-40 years, 41-60 years and ≥61 years.

The results obtained from this study showed a positive association of HbA1c with age in non-diabetics. The mean values of HbA1c were observed to significantly ($p<0.05$) increase with an increase in age (Table 1). This result is in agreement with the results of previous investigators [5, 6, 14-19]. The positive significant association of HbA1c with age observed in our study could be attributed to certain factors unrelated to glycaemia since the subjects have no glucose metabolic impairment. An example of non-glycaemic factors is the changes in the rate of glycosylation associated with the ageing process [14]. There is a usual tendency for the body's metabolic machinery to decrease in efficiency with ageing. It is documented that, the basal metabolic rate (BMR) usually decreases by 2% per decade of adult life [20]. Our results, however, differed with other previous studies that showed no association between age and HbA1c levels [7-9].

Secondly, this study showed no significant association between HbA1c and sex. However, slight differences were observed between the HbA1c values in men and that in women across the age groups (Table 2). The lower values of HbA1c observed in women compared to that in men may be due to differences in haemoglobin levels in men and women. Men and women usually have different mean haemoglobin levels in venous blood: women usually have mean levels approximately 12% lower than men [21]. The literature further indicates that the male sex hormone, testosterone, has a direct positive effect on erythropoietin and hence the red blood cell concentration [22, 23]. This really accounts for the higher haemoglobin content in healthy men compared to that in healthy women of same age and race. Also, evidence shows that the female hormone, oestrogen, is implicated in suppressing erythropoiesis in women *in vitro* [24]and *in vivo* [23, 25]. Hence lower haemoglobin content in women. Glycosylation begins during erythropoiesis and so is directly related to the amount of HbA1c that would be formed. Our result is similar to the studies by Faerch*etal,* and that of Gulliford and Ukoumunne. Both found somewhat higher levels of HbA1c in men compared to women which were not significant [26,27]. Likewise, Wiener and Roberts stated that they found no relationship between the levels of HbA1c with sex [8]. Modan*et al* and Simon *et al* out rightly stated that they found no association of HbA1c with gender [19, 28]. Despite this, other studies reported the significant positive relationship between HbA1c and sex [6, 14].

Thirdly, in our study, HbA1c levels positively correlated (p=0.01) with body mass index (BMI). An increase in BMI was accompanied by an increase in HbA1c level. This association remained even after adjusting for age. This is in agreement with the results of previous researchers. The study of Gulliford and Ukoumunne on the determinants of HbA1c in general population showed that Hba1c increased with BMI and with increasing waist-hip circumference ratio [28]. Yang *et al* and Pani*et al*, as well noted a positive association between the levels of HbA1c and BMI [14, 15]. Likewise, Simon *et al*, found higher levels of HbA1c in obese persons (defined as >28kg/m^2). However, after adjustment for age, the association became non-significant. On the contrary, Modan*etal,* found no significant correlation between BMI and HbA1c [28], while surprisingly, Shultis*etal* found suggestive evidence of inverse associations between body size and body composition and HbA1c [16].

The contribution of the present study to the existing literature lies on the fact that the subjects of this study were of the West African origin. To the best of our knowledge, there is no study on the effect of age, sex and BMI that has been carried out among the West African population. Likewise, this study made sure that all relevant age-groups were captured.

However, it is noteworthy to state that there are some limitations in this study. One was that the sample size was relatively small especially at the one extreme end of the age groups (i.e. the age group 'above 60 years old'). This was mainly due to the cultural superstition that is associated with the use of human samples (e.g. blood) as well as due to the invasiveness and the need for repeating the experiment.

5. Conclusion

In summary, our study showed that age and BMI positively associated with HbA1c in a non-diabetic Nigerian population. The positive association of HbA1c with age has clinical consequences. One such consequence is in the management of older diabetic patients who are usually prone to the risk of hypoglycaemia due to anti-diabetic drug overuse. Since certain factors unrelated to glycaemia may be contributing to increasing in HbA1c levels in non- diabetic normal glucose tolerant individuals as noted earlier, it goes to imply that the current HbA1c targets [American Diabetes Association, (HbA1c<7%) or the American College of Endocrinology (HbA1c ≤6.5%)] for diabetics, which did not take into account the contribution of age may need to be reviewed and the age factor taken into account in order to minimize the risk of hypoglycaemia and other medication side effects. This study also showed that there is no significant correlation between HbA1c and sex even though there was a certain association, with men having higher values than women.

Further studies are required especially to investigate the non-glycaemic factors affecting HbA1c levels in normal glucose tolerant populations so as to really understand the actual role glycosylated haemoglobin play in diabetes management and diagnosis.

REFERENCES

[1]. D. M.Nathan, J. Kuenen, R. Borg, H. Zheng, D.Schoenfeld, and R. J. Heine, A1c-derived average glucose study group. Translating the A1C assay into estimated average glucose values. *Diabetes Care,31*, 2008, 1473-1478.

[2]. B.K. Manjunatha, N. Bhava, D.O. Sarsina, T. G. Sathisha, S. Sweta and R.N. Devaki, Relation of calculated HbA1c with fasting plasma glucose and duration of diabetes. *International Journal of Applied Biology and Pharmaceutical Technology*, *2(2)*,201158-61.

[3]. C. Kim,K.M. Bullard,W.H. Herman, and G.L. Beckles, The association between iron deficiency and the HbA1c levels among adults without diabetes in the national health and nutrition examination survey, 1999–2006. *Diabetes Care*, *33,* 2010, 780–785.

[4]. B.B. Arnetz, A. Kallner, andT. Theorell, The influence of aging on HbA1c. *Journal of Gerontology,37,* 1982, 648-650.

[5]. E.S. Kilpatrick, M.H. Dominiczak, and M. Small, The effect of aging on glycation and the interpretation of glycemic control in type 2 diabetes. *Quatar Journal of Medicine,89,*1996, 307-312.

[6]. A.P. Yates, and I. Laing, Age-related increase in haemoglobin A1c and fasting plasma glucose is accompanied by a decrease in β cell function without change in insulin sensitivity: Evidence from a cross-sectional study of hospital personnel. *Diabetes Medicine,19,* 2002, 254-258.

[7]. U.M. Kabadi, Glycosylation of proteins: lack of influence of ageing. *Diabetes Care, 11*, 1998, 421-432.

[8]. K. Wiener, and N.B. Roberts, Age does not influence levels of HbA1c in normal subject. *Quatar Journal of Medicine, 92,* 1999, 169-173.

[9]. P.S. Vallee, V. Lasserre, M. Fonfrede, and S. Benazeth, A different approach to analyzing age-related HbA1c values in non-diabetic subjects. *Clinical Chemical Laboratory Medicine, 42,* 2004, 423-428.

[10]. P. Trinder, Quantitative determination of glucose using GOD-PAP method. *Annals Clinical Biochemistry.* 6, 1969, 24-47.

[11]. L.A. Trivelli, H.M. Ranney, and H.T. Lai, Hemoglobin components in patients with diabetes mellitus. *New England Journal of Medicine,284*, 1971, 353-357.

[12]. National Committee for Clinical Laboratory Standards, Procedure for Determining Packed Cell Volume by the Microhematocrit Method, 2nd (Edn.) H7-A2. Villanova Pa, 1993.

[13]. R.G. Schneider, Methods for detection of hemoglobin variants and hemoglobinopathies in the routine clinical laboratory. *Critical ReviewsinClinicalLaboratorySciences,* 1978.

[14]. L.N. Pani, L. Korenda, J.B. Meigs, C.Driver, S. Chamany, and C.S. Fox, Effect of aging on A1c levels in individuals without diabetes. *Diabetes care*, 31, 2008, 1991-1996.

[15]. Y.C. Yang, F.H. Lu,J.S. Wu, and C.J. Chang, Age and sex effects on HbA1c: A study of Chinese healthy population. *Diabetes Care,* 20, 1997, 988-991.

[16]. W.A. Shultis, S.D. Leary, A.R. Ness, J. Scott, R.M. Martin, and P.H. Whincup,Haemoglobin A1c is not a surrogate for glucose and nsulin measures for investigating the early life and childhood determinants of insulin resistance and Type 2 diabetes in healthy children. An analysis from the Avon Longitudinal Study of Parents and Children (ALSPAC). *DiabetesMedicine*, ***23****(12)*, 2006, 1357-1363.

[17]. L. Chi-chang, T. Kun-wu, M. Shih-ming, C. Shan-fan, and W. Chin-chu, The relationship between fasting glucose and HbA1c among customers of health examination services. *Formos Journal of Endocrine Metabolism,1(3),* 2010, 9-13.

[18]. M.B. Davidson, and D.L. Schriger, Effect of age and race/ethnicity on HbA1c levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. *DiabetesResClinicalPractice*, *87(3)*, 2010, 415-421.

[19]. D. Simon, C. Senan, P. Garnier, M. Saint-Paul, andL. Papoz, Epidemiological features of glycated haemoglobin A1c distribution in a healthy population: the Telecom Study. *Diabetologia, 32*, 1989, 864 – 869.

[20]. S.G. Chaney, Principles of Nutrition I: Macronutients. In: Devlin, M.T. (Ed.). T*extbook of Biochemistry with Clinical Correlations* 6th (Edn),(Hoboken, NJ: Wiley-Liss, 2006)1072.

[21]. V. Ganji, and M.R. Kafai, Hemoglobin and hematocrit values are higher and prevalence of anemia is lower in the post-folic acid fortification period than in the pre-folic acid fortification in US adults. *American Journal of Clinical nutrition, 89*, 2009, 363-371.

[22]. S. Shahani, M. Braga-Basaria, M. Maggio, and S. Basaria, Androgens and erythropoiesis: past and present. *Journal of Endocrinological Investigation,32,*2009, 704-716.

[23]. W. Jelkmann, Regulation of erythropoietin production. *Journal of Physiology,598,*2011, 1252-1258.

[24]. G.A. Blobel, andS.H. Orkin,Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GAT A-1. *Molecular cell Biology, 16*, 19961, 687-1694.

[25]. P.P. Duke, and E. Goldwasser, Inhibition of erythropiesisbyestrogens. *Endocrinology, 69,* 1961, 21-29.

[26]. K. Faerch,K. Borch-Johnsen,A. Vaag, T. Jorgensen, and D.R. Witte, Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia*, *53(5)*, 2010, 858-865.

[27]. M.C. Gulliford, andO.C. Ukoumunne, Determinants of glycated haemoglobin in the general population: associations with diet, alcohol and cigarette smoking. *EuropeanJournal ofClinicalNutrition,55(7)*, 2001, 615-623.

[28]. M. Modan, D. Meytes, P. Rozeman, S.B. Yosef, E. Sehayek, and N.B. Yosef, Significance of high HbA1 levels in normal glucose tolerance. *DiabetesCare*, *11(5),* 1988, 422-428.