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Research Article

Association Between Haemoglobin Levels and Glycated Hemoglobin (Hba1c) in Diabetic Patients: A Case Control Study at Pentecost Hospital, Ghana

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Abstract

Background: Evaluation of HbA1c in diabetics has been found to be disconcerted by factitious results in anaemic diabetics. The degree of effect has however not been well established.

Aim: The primary objective of this research was to examine how changes in haemoglobin concentration impact HbA1c levels.

Methods: This study involved a hospital-based case-control approach, encompassing a total of 200 participants. The cohort included 100 diabetic and 100 non-diabetic patients, all of whom were receiving healthcare services at Pentecost Hospital. Convenience sampling method was employed. A 5 mL venous blood sample was drawn from each participant under aseptic circumstances. Following this, 2 mL of the venous blood was dispensed into fluoride oxalate tubes for the assessment of glucose levels, while the remaining 3 mL was carefully transferred into Ethylenediamine Tetra Acetic acid (EDTA) tubes to facilitate a comprehensive analysis of full blood count and HbA1c levels. The data collected was analyzed using Microsoft Excel 365 and SPSS software version 25.

Results: In the cohort of diabetic participants, a statistically significant linear relationship was established between the degree of anaemia and HbA1c levels, as indicated by a p-value less than 0.001. Concurrently, non-diabetic subjects demonstrated a statistically significant linear trend in HbA1c, also evidenced by a p-value less than 0.001. Additionally, a p-value of 0.002 indicated a statistically significant association between glycated Haemoglobin (HbA1c) and fasting plasma glucose levels among the diabetic subgroup. Conversely, this correlation failed to attain statistical significance among non-diabetic individuals, with a p-value of 0.690.

Conclusion: Monitoring diabetics solely based on HbA1c may be deceptive. It is recommended that clinicians exercise prudence by incorporating the anaemic status of diabetic patients into their therapeutic decision-making processes, particularly when relying exclusively on HbA1c assessments.

Keywords: Haemoglobin; HbA1c; Anaemia; Diabetes mellitus

Introduction

Diabetes mellitus is on the rise globally. According to estimates provided by the International Diabetes Federation (IDF), 537 million persons worldwide have diabetes in 2021.This projection indicates that the figure is expected to reach 643 million by 2030 and escalate further to 783 million by 2045. The glucose-haemoglobin complex known as glycated Haemoglobin (HbA1c) is created when hemoglobin undergoes irreversible, non-enzymatic glycation. When glucose attaches itself to the N-terminal valine residue on the beta chain of hemoglobin, the process known as glycation produces HbA1c. Haemoglobin A1c (HbA1c) is a common test for diabetes diagnosis, monitoring and management. The benefits of HbA1c over other glycemic control monitoring parameters include its stability in samples and the needlessness of fasting before the test Regardless of its

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advantages, HbA1c can be influenced by genetic, physiological, haematological, and other pathological variables. Conditions that affect haemoglobin, RBC turnover, and haemoglobin glycation can affect HbA1c levels independently of blood glucose. Since the HbA1c value represents the portion of total haemoglobin that is glycated, it is possible that the haemoglobin level influences HbA1c test results separately from glycemia. Several studies including those by [4,10,11] have been conducted to study the correlation between haemoglobin and HbA1c in diabetes mellitus. Nevertheless, there is inconsistent reporting on how anemia affects HbA1c in these investigations. Anaemia may result in an incorrect rise or fall in HbA1c concentration, misrepresenting the state of the disease. There is also dearth of information on the relationship between HbA1c and anaemic status in Ghana. In this study, it was hypothesized that HbA1c was lower in diabetic patients who are anaemic.

Materials and Methods

Study Design

This study examined the relationship between hemoglobin levels and HbA1c concentration in individuals with and without diabetes at the Pentecost Hospital from June 2022 to August 2022. It was a case control investigation.

Participants

The study population consisted of 100 diabetics who attended the Pentecost hospital diabetic clinic and 100 non-diabetic patients who presented at the Outpatients' Department of the Pentecost Hospital between June and August 2022. The sample size was calculated using the Cochran's equation, with a diabetes prevalence of 6.46%, a confidence level of 95% and an error margin of 5%. Adult diabetics (type 1 and 2) who have been managed for diabetes at the Pentecost hospital clinic for at least two years were included in the study. Patients taking any type of oral iron supplements, acute diabetic complications, recent history of surgery, those who have had a recent accident or trauma leading to acute haemorrhage, those who have had a recent blood transfusion and pregnant patients were not included in the study.

Methods

Five milliliters (5ml) of blood were obtained using a 5cc 21-gauge hypodermic syringe. 2ml of the blood was transferred into fluoride oxalate tubes and the remaining 3ml was transferred into EDTA tubes. The test tube was labelled appropriately with the name and ID number given to the participant during the research. The EDTA anticoagulated blood was tested for full blood count to obtain haemoglobin concentration using Urit 5250 fully automated haematology analyzer (Medtek, China) and HbA1c using Finecare™ FIA Meter Plus (Wondfo, China). After centrifugation, the plasma from the fluoride oxalate tubes were analyzed for glucose estimation using Mindray BS 430 chemistry analyzer (Mindray, China). Haemoglobin level was classified as normal (males -13 to 17g/dL, females - 12 to 16g/dL), mild anaemia (males - 10 to 12.9g/dL, females – 10 to 11.9g/dL), mild anaemia (males – 8 to 9.9g/dL, females – 8 to 9.9g/dL) and extreme anaemia (males - <7.9g/dL, females - <7.9g/dL).

Statistical Analysis

Descriptive statistics, encompassing measures such as frequency, percentage, range, mean, and standard deviation, were employed for comprehensive data characterization of the sex-wise distribution of anaemia in participants and the dependent variable (HbA1c). The relationship and degree of the association between haemoglobin level and HbA1c were examined using scatter plots, Pearson correlation and regression analysis. If the p-value was less than 0.05, the statistical significance criterion was deemed significant.

Results

General Characteristics of Study Participants

Table 1 displays the descriptive statistics for the study sample, providing an overview of the key characteristics of the participants. The diabetic participants were significantly older (59.03±14.78 years) than the non-diabetic participants (49.81±16.28 years, *P<.001*). No significant difference was found between the haemoglobin level in diabetics (11.91±2.41g/dL) and non-diabetics (12.36±2.30g/dl, *P=.181*), although the non-diabetic participants had a slightly higher haemoglobin level than the diabetics.

Comparison of Anaemia Severity by mean Haemoglobin among Study Participants

The anaemic participants were grouped according to their haemoglobin measures into mild anaemia (males - 10 to 12.9g/dL, females – 10 to 11.9g/dL), mild anaemia (males – 8 to 9.9g/dL, females – 8 to 9.9g/dL) and extreme anaemia (males - <7.9g/dL, females - <7.9g/dL). Table 2 shows the mean hemoglobin levels for the individuals with diabetes and those without the disease. Comparison of the mean haemoglobin of mildly anaemic diabetic (11.19±0.94g/dL) and non-diabetic (11.38±0.72g/dL) participants showed no significant difference (*P=.395*). The mean haemoglobin of moderately anaemic diabetic (9.26±0.28g/dL) values did not exhibit any statistically significant difference from non-diabetic (9.10±0.52g/dL) participants (*P=.424*). The mean hemoglobin of diabetic patients with extreme anaemia did not differ significantly (6.24±1.04g/dL) and non-diabetic (6.47±1.22g/dL) participants *(P=.728)*.

Correlation between FBG and HbA1c Among Study Participants

Figure 1 shows the association between FBG and HbA1c in diabetic and non-diabetic participants. From figure 1a shows no relationship between FBG and HbA1c in the non-diabetic participants (*P=.690*). Conversely, figure 1b demonstrates a statistically significant positive correlation (P=.002) between FBG and HbA1c in the people with diabetes.

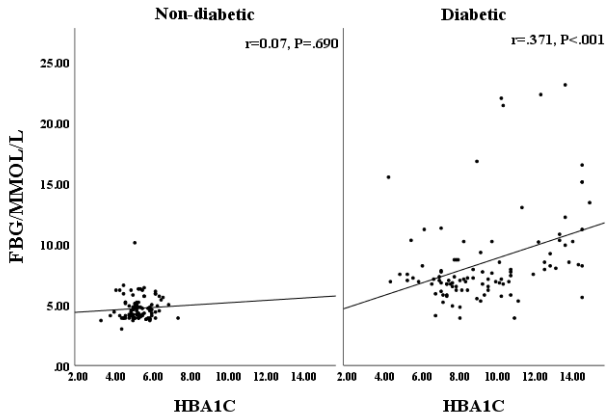

Figure 1: Correlation between FBG in diabetic and non-diabetic participants.

HbA1c Trend based on Anaemia Sseverity Among Study Participants

A line graph in Figure 2 illustrates how the degree of anemia affects HbA1c in both diabetic and non-diabetic individuals. HbA1c was highest in the non-anaemic group (10.18±2.28%) among the diabetic patients and declined steadily as anemia level rose; however, this trend did not reach statistical significance in the mild and severe anaemic groups. The group of non-diabetic subjects with slightly anemia had the greatest HbA1c (5.39±0.73%), and it dropped as the severity of anemia increased. The statistical significance of the trend in glycated haemoglobin (HbA1c) levels across different degrees of anemia across both diabetes and non-diabetic cohorts was evaluated using a one-way Analysis of Variance (ANOVA). Among the diabetic patients, the HbA1c concentration significantly decreased as the degree of anemia rose (P<.001). The HbA1c concentration significantly decreased as the degree of anemia rose in the non-diabetic subjects as well (P<.001).

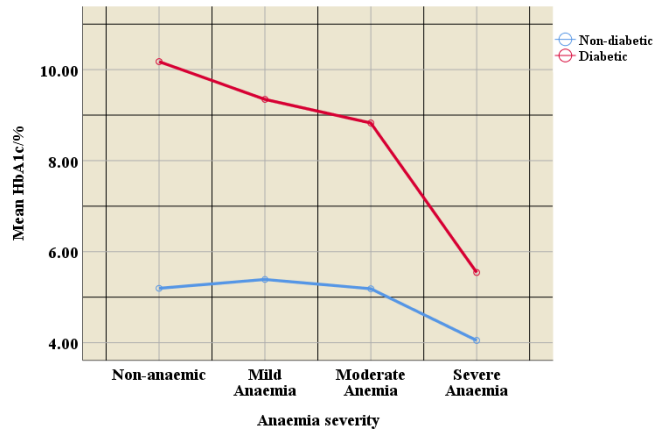

Figure 2: HbA1c trend in anaemia severity among diabetic and non-diabetic participants.

Table 1: General characteristics of the study participants.

Variables	Non-diabetics (N=100)	Diabetics (N=100)	*P-value*
Sex			
Male	37(37%)	36(36%)	0.883
Female	63(63)	64(64%)	
Age/ years	49.81±16.28	69.03±14.78	<.001*
FBG/ mmol/L	4.71±0.97	8.52±3.80	<.001*
HbA1c/ %	6.18±0.68	9.46±2.61	<.001*
Hb / g/dl	12.36±2.30	11.91±2.41	0.181
Red cell Indices			
MCV/fL	79.88±6.05	78.01±7.28	0.051
MCH/pg	27.34±2.98	27.13±2.90	0.636
MCHC/g/dL	33.80±2.28	33.92±2.24	0.699
Anaemia classification			
Normal	59(59%)	52(52%)	0.688
Mild anaemia	28(29%)	30(30%)	
Moderate anaemia	7(6%)	11(11%)	
Severe anaemia	6(6%)	7(7%)	

For continuous data, the data was presented as mean ± SD, and for categorical data, as %. The acronyms Hb, MCV, MCH, MCHC, FBG, and HbA1c stand for hemoglobin, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, Fasting Blood Glucose, Haemoglobin A1c. The student t test and the Chi-squared test were used to determine the P-value, respectively.

Table 2: Comparison of anaemia severity by mean Hb among diabetic and non-diabetic study participants.

	Diabetic status			*P-value*
	Non-diabetic	Diabetic	T-stat	
Normal	13.82±1.07	13.67±1.15	0.745	0.458
Mild	11.38±0.72	11.19±0.94	0.856	0.395
Moderate anaemia	9.10±0.52	9.26±0.28	-0.821	0.424
Severe anaemia	6.47±1.22	6.24±1.04	0.357	0.728

Table 3: Multiple regression analysis with HbA1c as the dependent variable and Hb, FBG and age as the independent variables.

Independent variable	Non-diabetics		Diabetics	
	B	*P-value*	B	*P-value*
Constant	3.882	*<.001*	-0.064	*.971*
Hb/g/dL	0.099	*<.001*	0.572	*<.001*
FBG/mmol/L	0.040	*.301*	0.233	*<.001*
Age	0.008	*.768*	0.013	*.391*

a. Dependent Variable: HbA1c/%

* The variables in the model explained around 44.5% of the variability observed in HbA1c levels (total R2=0.445). The estimated change in HbA1c for a one-unit change in the independent variables is shown by the regression coefficient (B).

Linear Regression of Study Variables Against HbA1c in Study Participants

Using data from Hb, FBG, and age, a multiple regression analysis was performed to predict HbA1c in study participants who were either diabetic or not. Regression analysis showed a positive coefficient estimate for the link between Hb and HbA1c among the non-diabetic subjects, indicating a positive linear relationship between the two variables (B=0.099, P<.001; Table 3). Regression analysis revealed non-significant relationships in non-diabetic persons between age and glycated hemoglobin (HbA1c) (B=0.008, P=.768) and between Fasting Blood Glucose (FBG) and HbA1c (B=0.04, P=.301) (Table 4). Additionally, FBG and HbA1c had a significant relationship (B=0.233, P<.001). However, among individuals with diabetes, age did not significantly correlate with HbA1c (B=0.013, P=.391; Table 3).

Discussions

Despite ongoing discussions regarding its relevance for diagnosis, HbA1c is still not an authenticated instrument for the diagnosis of diabetes mellitus. Anaemia can either raise or lower HbA1c levels due to variations in RBC half-life. This investigation was undertaken with the objective of evaluating the correlation between hemoglobin levels and HbA1c among individuals with diabetes, building upon this underlying premise. This current study demonstrated a significantly lower HbA1c concentration in anaemic diabetic and non-diabetic participants. Studies conducted in 2014, 2015, and 2019 by Kalasker et al., Cavagnolli et al., and Solomon et al., respectively, all lend credence to this. Conversely, these three studies contradicted previous research because the association between hemoglobin and HbA1c has either been reversed or not detected in other studies. Nonetheless, these results suggest that anemia may affect HbA1c readings, which could result in inaccurate interpretations, particularly in those with diabetes. The glycation process is impacted by changes in erythrocyte lifespan and turnover, which can affect HbA1c values in anemia, a disorder characterized by insufficient red blood cells or hemoglobin levels in the bloodstream. As a result, using HbA1c alone to assess glycemic control might not provide an accurate representation in individuals with concomitant anaemia, particularly in diabetic patients. According to a 2017 study by Alsayegh et al., anemia is significantly more common in diabetic patients (females > males), with the majority of cases being related to diabetic complications. However,

it did not show a connection between HbA1c and anemia in either male or female. The researcher went on to say that anemia in diabetics needs to be identified and treated right away because it is associated with major complications from the disease. Higher HbA1c readings in anemia were found in studies conducted by [22,23].

As HbA1c is expressed as a percentage of total hemoglobin A, [24] suggested that a decrease in hemoglobin concentration could result in an increase in the glycated part at a constant glucose level. According to [6], variations in the laboratory methods used for measuring HbA1c may also be to blame for reported variances in HbA1c concentrations between anemic and non-anemic individuals. Research has made an effort to clarify how the Hb level affects the HbA1c. In a specific study conducted in 2016, [26] found that among 122 participants (67 with anemia and 67 without anemia), the HbA1c level did not significantly change based on the measurement method (such as High-Performance Liquid Chromatography (HPLC) method and immunoturbidimetry method; P=0.192). Mild anemia has little impact on HbA1c, but moderate to severe anemia has been shown to increase it. The HbA1c test can be used to diagnose diabetes in people who have mild anemia. The relationship between anemia and HbA1c level is not clearly defined; the cause and effect of anemia are complex. In [28] emphasized the significance of additional research.

Only those with diabetes had a substantial FBG-HbA1c correlation; non-diabetics did not. This result is consistent with studies conducted in 2016 by [29] and 2020 by [4]. The substantial correlation between HbA1c and fasting plasma glucose that has only been seen in diabetics implies that the existence of anemia may have an impact on the link between these two indicators. The difference in the connection between fasting plasma glucose and HbA1c between persons with and without diabetes highlights the need for caution when interpreting HbA1c results, especially in cases where anemia is present. It is interesting to note that across patients with and without diabetes, age did not significantly predict HbA1c. Age is a factor that is frequently considered when managing diabetes since glycemic control may be predicted to fluctuate as one ages due to changes in metabolic processes and the possible effects of aging on general health. The findings of this study, however, imply that age may not be a significant factor in determining HbA1c levels in those with or without diabetes. This result was similarly consistent with a 1999 study conducted by [30]. To put it succinctly, the only markers of HbA1c that were significantly correlated with it were Hb and FBG.

Conclusion

In this study, we found that haemoglobin levels in both diabetic and non-diabetics were positively and significantly associated with HbA1c. With increasing degree of anaemia severity, HbA1c decreases. This erroneous decrease in HbA1c may cause diabetes to be underdiagnosed and may also give a false sense of glycaemic control in known diabetics. Due to this, the study emphasises how crucial it is to exclude anaemia before making any clinical decisions dependent on HbA1c levels.

Author Statements

Ethical Considerations

In addition to verbal assent from the participants, ethical approval was received from the Research and Ethical Review committee of the Department of Medical Laboratory Technology of Accra Technical University (Reference No. ATU/MLT/ET/01180010B/2021-2022).

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Author contributions

MA and FA came up with the project's core concepts. The study's design involved both MA and FA. FA wrote the original draft after studying the data. MA, EN, SA, EKA, SL and RDT gave the paper a careful evaluation before deciding whether to submit it for publication. The completed manuscript was given their respective approvals before submission.

Institutional Review Board Statement

The research was carried out in compliance with the Accra Technical University Declaration and authorized on June 8, 2022, by the Accra Technical University Research Ethics Committee (Reference No. ATU/MLT/ET/01180010B/2021-2022).

Informed Consent Statement

Every participant in the study gave their verbal consent.

Conflicts of Interest

There are no conflicts of interest reported by the writers.

References

1. Lin X. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Scientific Reports. 2020; 10: 14790.
2. Federation ID. IDF Diabetes Atlas | Tenth Edition. In: Diabetes around the world in 2021. 2021.
3. Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM. Glycosylated haemoglobins and long-term blood glucose control in diabetes mellitus. J Clin Endocrinol Metab. 1977; 44: 859-864.
4. Narayanan S, Dash P, Mahajan P. Effect of total haemoglobin level on hba1c value in type 2 diabetes mellitus patients. Bangladesh Journal of Medical Science. 2020; 19: 110-113.
5. Association AD. Standards of Medical Care in Diabetes. Diabetes Care. 2009; 32: s13-s61.
6. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A New Look at Screening and Diagnosing Diabetes Mellitus. J Clin Endocrinol Metab. 2008; 93: 2447-2453.
7. Cohen RM. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood. 2008; 112: 4284-4291.
8. Dagogo-Jack S. Pitfalls in the use of HbA1(c) as a diagnostic test: the ethnic conundrum. Nat Rev Endocrinol. 2010; 6: 589-593.
9. Bae JC. Haemoglobin A1c values are affected by haemoglobin level and gender in non-anaemic Koreans. J Diabetes Investig. 2014; 5: 60-65.
10. Takeuchi M, Kawakami K. Association between Haemoglobin and Haemoglobin A1c: A Data-Driven Analysis of Health Check-up Data in Japan. J Clin Med. 2018; 7: 539.

11. Solomon A, Hussein M, Negash M, Ahmed A, Bekele F, Kahase D. Effect of iron deficiency anaemia on HbA1c in diabetic patients at Tikur Anbessa specialized teaching hospital, Addis Ababa Ethiopia. BMC Hematol. 2019; 19: 2.

12. Sinha N, Mishra TK, Singh T, Gupta N. Effect of Iron Deficiency Anaemia on Haemoglobin A1c Levels. Ann Lab Med. 2012; 32: 17-22.

13. Katwal PC, Jirjees S, Htun ZM, Aldawudi I, Khan S. The Effect of Anaemia and the Goal of Optimal HbA1c Control in Diabetes and Non-Diabetes. Cureus. 2020; 12.

14. Asamoah-boaheng M, Sarfo-kantanka O, Tuffour AB, Eghan B, Mbanya JC. Prevalence and risk factors for diabetes mellitus among adults in Ghana: a systematic review and meta-analysis. In: no. 2018; 11: 83-92.

15. Martinsson A, Andersson C, Andell P, Koul S, Engström G, Smith JG. Anaemia in the general population: Prevalence, clinical correlates and prognostic impact. Eur J Epidemiol. 2014; 29: 489-498.

16. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Iris Institutional repository for Information Sharing. 2021.

17. Basit A, Fawwad A, Basit KA, Waris N, Tahir B, Siddiqui IA. Glycated haemoglobin (HbA1c) as diagnostic criteria for diabetes: the optimal cut-off points values for the Pakistani population; a study from second National Diabetes Survey of Pakistan (NDSP) 2016–2017. BMJ Open Diabetes Res Care. 2020; 8: 001058.

18. Kalasker V, Sudhamadhuri, Kodilwadmath M, Bhat H. Effect of iron deficiency anaemia on glycosylated haemoglobin levels in non-diabetic Indian adults. International Journal of Medical and Health Sciences. 2014; 3: 40-43.

19. Cavagnolli G, Pimentel AL, Freitas PAC, Gross JL, Camargo JL. Factors affecting A1C in non-diabetic individuals: Review and meta-analysis. Clinica Chimica Acta. 2015; 445: 107-114.

20. Miedema K. Standardization of HbA1c and Optimal Range of Monitoring. Scand J Clin Lab Invest. 2005; 240: 61-72.

21. Alsayegh F, Waheedi M, Bayoud T, Hubail A, Al-Refaei F, Sharma P. Anaemia in diabetes: Experience of a single treatment center in Kuwait. Prim Care Diabetes. 2017; 11: 383-388.

22. Makadia MG. Study of Glycated Haemoglobin (HbA1c) In Non-Diabetic Subjects with Subclinical Hypothyroidism. J Clin Diagn Res. 2017; 11.

23. Bindayel IA. Influence of iron deficiency anaemia on glycated haemoglobin levels in non-diabetic Saudi women. Journal of International Medical Research. 2021; 49: 1-10.

24. El-Agouza I, Shahla AA, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol. 2002; 24: 285-289.

25. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008; 93: 2447-2453.

26. Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Clin Biochem. 2016; 49: 117-120.

27. Inada S, Koga M. HbA1c and Glycated Albumin Levels Are High in Gastrectomized Subjects with Iron-Deficiency Anaemia - PubMed. Ann Clin Lab Sci. 2017; 47: 52-57.

28. Guo W, Zhou Q, Jia Y, Xu J. Increased Levels of Glycated Haemoglobin A1c and Iron Deficiency Anaemia: A Review. Med Sci Monit. 2019; 25: 8371-8378.

29. Stamouli M, Pouliakis A, Mourtzikou A, Skliris A, Panagiotou I. Identifying the Relation between Fasting Blood Glucose and Glycosylated Haemoglobin Levels in Greek Diabetic Patients. Annals of Cytology and Pathology. 2016; 1: 025-033.

30. Wiener K, Roberts NB. Age does not influence levels of HbA1c in normal subject. QJM: An International Journal of Medicine. 1999; 92: 169-173.