Reference Ranges for Glycosylated Haemoglobin And The Correlation Between Glycosylated Haemoglobin Levels And Random Blood Glucose, Hemoglobin And It's Related Red Blood Cell Indices

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Abstract: A reference value for glycosylated haemoglobin in the Kenyan population in Kiambu County was developed. 600 normal adult individuals were recruited in the study, 7 (1.2%) of their sample were not analysed as they were positive for HIV (5) and HbsAg (2). The remaining 593 study participants had representation of male 325 (54.8%) and female 268 (45.2%).Six millitres of blood was obtained from the participants three millitres put in EDTA vacuitainer for analysis of glycosylated haemoglobin and three millilitres put in a plain vacuitainer for screening of HIV, HbsAg and VDRL.Statistical package for the social sciences (SPSS) programme (version 21) was used for data analysis. T-test was used to compare the levels of the measured parameters between sexes, ANOVA and post ANOVA was used to compare the value of each parameter across the various age groups. P<0.05 was considered significant.Means difference between male and female for analysed glycosylated haemoglobin parameter was statistically significant (p < .003.) and therefore separate reference ranges were established for adult population in Kiambu county. Mean and standard deviation (SD) for the studied male population was 4.57 and 0.90 respectively. Using the formula; Mean +/- 1.96 SD lower reference value was found to be 2.8 % and the high reference value to be 6.4. Mean and standard deviation (SD) for the studied female population was 4.34 and 0.92 respectively. Using the formula; Mean +/- 1.96 SD hence lower reference value was found to be 2.5 % and the high reference value to be 6.2 %. 323 diabetic patients attending diabetic clinic at Thika level five hospital were recruited to assess the correlation of glycosylated hemoglobin with random blood sugar, hemoglobin, packed cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume. For the diabetic patients all the recruited study populations were included in the study. Four millitres of blood sample was obtained from the patients and was put in EDTA vacuitainer. The sample was used for analysis of glycosylated haemoglobin and the full haemoglobin. Pearson's product moment correlation matrix with the dependent and independent variables was performed to assess the strength and direction of the associations between the variables of interest. Four factors especially correlated with the dependent variable glycosylated hemoglobin: hemoglobin (r = .467, ρ = .000), packed cell volume (r = .435, ρ = .000), mean cell hemoglobin (r = -.165, ρ = .003), and random blood glucose (r = .626, ρ = .006). This meant that an increase in random blood glucose, hemoglobin, packed cell volume, and mean cell hemoglobin, increases glycosylated hemoglobin. Mean cell haemoglobin concentration and mean cell volume had no significant correlation with glycosylated haemoglobin. A multiple linear regression analysis was applied to explain the direction and strength of relationships between the dependent variable glycosylated hemoglobin and independent variables, random blood glucose, haemoglobin, packed cell volume, mean cell haemoglobin, mean cell hemoglobin concentration, and mean cell volume. The coefficient of determination R^2 in this model equation was found to be .552, which meant that the six independent variables explained 55.2% of the variation of glycosylated hemoglobin. The other 44.8% of the variation being accounted for by factors other than those included in this study.

Key words: correlation, glycosylated haemoglobin, reference ranges, Kiambu County.

INTRODUCTION

Glycosylated hemoglobin (HbA1c) is the hemoglobin to which glucose is attached to the haem part of the red blood cell. It is the type of hemoglobin that is analysed to help determine the mean blood glucose levels over a given period of time of about three to four months. Increased level of plasma glucose leads to increased amount of glycosylated hemoglobin. In diabetes mellitus, increased

glycosylated hemoglobin levels, indicates poor management of hyperglycemia. This has been associated with conditions such as kidney failure, retinopathy nerve damage and cardiovascular disease (Sidorenkov et al., 2011)

Prior to the last two decades of the twentieth century, Clinical Chemistry Laboratory qualitative results were interpreted by comparison to tradition but inadequately defined "normal ranges" often used to characterize the values of health subject. The term "normal" was confusing in that in the context of Clinical Chemistry, it was assumed to present the normal (healthy) subject and to signify the normal (Gaussian) distribution. However, increasing awareness of the biological changes in physiological processes demands precise and comprehensive interpretation. A lot of review and recommendation have facilitated the abolishment of "normal" ranges to introduction of reference ranges (Grasbeck, 1969; Martin *et al*., 1975; Galen, 1977).

Currently there are no reference values for glycosylated hemoglobin in health institutions in Kiambu County which are based on the healthy local population who seek medical care from these institutions. Health providers use glycosylated hemoglobin reference ranges quoted from diagnostic reagent kits which represent population from other geographical location to manage diabetes mellitus in the county health institutions. The purpose of the study was to develop reference ranges for Glycosylated hemoglobin for health population to be used by Clinical Chemistry Laboratories in health institutions in the County of Kiambu, Kenya and to establish if there is any significant differences between the obtained values for this parameter in the study population of Kiambu County and those provided in reagent manufacturer's literature. The study was also designed to establish if there is correlation of glycosylated hemoglobin levels with random blood glucose, hemoglobin and its related red blood indices.

MATERIALS AND METHODS

Study Period: July 2016 to December 2016.
Study site: The study was carried out at Thika Level Five Hospital, The main analytical centers were the Laboratory Medicine department, Kenyatta National Hospital and Jomo Kenyatta University of Agriculture and Technology, Medical Laboratory Sciences department, Hematology and Virology laboratories.
Study population: Five hundred and ninety three health individuals comprising of 325 male and 268 female were recruited into the study for establishment of the reference ranges. Three hundred and twenty two diabetic patients attending diabetic clinic at Thika Level five hospital were also recruited for establishment of correlation of glycosylated hemoglobin levels with random blood glucose levels, hemoglobin and its related hematological indices levels. The participants aged between 18-60 years.
Blood specimen collection: Four millilitres of venous blood were collected from the diabetic patients. The samples were put in a 5 millilitres Ethylene Diamine tetra-acetic acid (EDTA) vacutainers and then properly mixed with the anticoagulant by gently swirling the tube for about 30 seconds. Samples were used for analysis of glycosylated haemoglobin and full haemogram. Six milliliters of blood was collected from the health population, 3 millilitres of the sample was put in an EDTA tubes well mixed for analysis of glycosylated haemoglobin and 3 millilitres put in a plain tube. These were left to clot and separated by centrifuging at 3000g for 5 minutes. The samples were used to screen for Hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV), Syphilis (VDRL), Hepatitis C virus (HCV), and pregnancy in females.
Sample Analysis: Glycosylated haemogram was analysed using midray 800 auto analyser (Germany). Auto Hematology Analyzer (Nanjing Perlove Medical equipment Co, Ltd, China) was used to analyse full haemoglobin and random blood glucose was analysed using expeed glucometer in combination with glucose electrode. For HIV, HbsAg, HCV, VDRL and pregnancy tests rapid screening tests were done using an Immunochromatographic reagent strip Quality control samples were run always before analysis of the study samples.
Data Analysis: Version 21 of the **S**tatistical package for the social sciences (SPSS) programme was the tool applied to analyse the results. T-test was used to compare the levels of the measured parameters between sexes, ANOVA and post ANOVA was used to compare the value of each parameter across the various age groups. $P<0.05$ was considered significant. The Pearson's product moment correlation analysis measured the associations between the dependent and the independent variables and Multiple Linear Regression was used to determine the strength of those factors affecting glycosylated hemoglobin.

RESULTS

The reference values were determined using the means and standard deviations as the data was found to be normally distributed. The means difference for both sexes were statistically compared using t- test as shown in table1. They were statistically significant since ρ was less than 0.05 and hence the reference ranges were established for each individual sex. Using the formulae Mean±1.96 SD, the reference ranges for male (Mean= 4.57, S.D = 0.91) were established lower limit 2.8% and higher limit 6.4%. For the female (Mean= 4.34, S.D = 0.92) the reference limits were established lower limit 2.6% higher limit 6.2%.

Table 1: Established reference ranges for the HbA1c for both male and females

Gender	N	Mean±SD	Mean-1.96SD	Mean+1.96SD	Range	t-value	ρ-value

M &F	593	4.48±0.98	2.56	6.40	3.84	3.000	0.003
M	325	4.57±0.91	2.79	6.35	3.56		
F	268	4.34±0.92	2.53	6.15	3.62		

N stands for number of the subjects, SD = Standard difference.

Using the T- test the means difference for the two sexes within the same age groups for all the age categories (18-29 years, 30-39 years, 40-49 years and 50 and above years) were not statistically significant. Using ANOVA the means difference of the analyte in the four categories for the male was statistically significant ($\rho = 0.006$) whereas the means difference was not statistically significant in the case of the females within the four categories ($\rho = 0.851$). Using the post hoc test in males, the means difference between category 1 and 2, category 1 and 3, category 1 and 4 were statistically significant ($\rho = 0.005$, $\rho = 0.004$ and $\rho = 0.005$), respectively. The means difference between category 2 and 3, category 2 and 4 and category 3 and 4 were not statistically significant ($\rho = 0.684$, $\rho = 0.506$, $\rho = 0.769$), respectively as shown in table 2.

Table 2 Comparison of means differences between age and sex

AGE GROUPS	18-29 Years Mean±SD	N	30-39years Mean±SD	N	40-49years Mean±SD	N	≥50years Mean±**SD**	N
F &M	4.35±0.77	196	4.52±1.03	187	4.55±1.22	125	4.57±0.95	85
Female	4.42±0.93	104	4.29±1.00	71	4.31±1.14	53	4.34±0.85	40
Male	4.28±0.54	92	4.66±1.02[a]	116	4.72±1.26[b]	72	4.77±1.00[c]	45

a indicates significant means difference between age group 1 and age group2 in male where $\rho < 0.05$
b indicates significant means difference between age group 1 and age group 3 in male where $\rho < 0.05$
c indicates a significant means difference between age group 1 and age group 4 in male where $\rho < 0.05$

For the there hundred and twenty two samples analysed from diabetic patients, the associations between the variables were assessed using the Pearson's product moment correlation. The predictive power of the factors affecting glycosylated hemoglobin was determined by use of Multiple Linear Regression.

Correlation Matrix: The Pearson's product moment correlation matrix with the dependent and independent variables enabled the study to test the strength and direction of the relationships between the variables under study. Four factors correlated with the dependent variable glycosylated hemoglobin: hemoglobin ($r = .467$, $\rho = .000$), packed cell volume ($r = .435$, $\rho = .000$), mean cell hemoglobin ($r = -.165$, $\rho = .003$), and random blood glucose ($r = .626$, $\rho = .006$) as shown in table 3.

Table 3 Results of the Correlation Matrix

		HbA1c	Hb	PCV	MCH	MCHC	MCV	RBG
HbAlc	r	1						
	ρ							
	N	322						
Hb	r	.469**	1					
	ρ	.000						
	N	322	322					
PCV	r	.435**	.982**	1				
	P	.000	.000					
	N	322	322	322				
MCH	r	-.165**	-.099	-.112*	1			
	ρ	.003	.075	.045				
	N	322	322	322	322			
MCHC	r	-.044	-.034	-.061	.319**	1		
	ρ	.431	.539	.273	.000			
	N	322	322	322	322	322		
MCV	r	.084	.321**	.314**	.114*	.059	1	
	ρ	.135	.000	.000	.041	.289		
	N	322	322	322	322	322	322	
RBG	r	.626**	.223**	.217**	-.076	-.056	.214**	1
	P	.000	.000	.000	.173	.319	.000	
	N	322	322	322	322	322	322	322

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Multiple Linear Regression Analysis A multiple linear regression was used to explain the direction and strength of associations between the outcome variable glycosylated hemoglobin (HbAlc) and predictor variables, random blood glucose (RBG), hemoglobin (Hb), packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and mean cell volume (MCV) as shown in table 4. The Multiple Linear Regression analysis was used to test the hypotheses that the six predictor variables, random blood glucose, hemoglobin, packed cell volume, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration could significantly predict the outcome variable, the level of glycosylated hemoglobin

Table 4 Results for Multiple Linear Regression Analysis

Model	R	R square	Adjusted R square	Change Statistics				
				R square Change	F. Change	*df1*	*df2*	*Sig. F* Change
1	.773	.552	0.543	.552	64.471	6	314	.000

The coefficient of determination R^2 from the analysis was found to be .552. This meant that the six independent variables (random blood glucose, hemoglobin, packed cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume) explained 55.2% of the variation of glycosylated hemoglobin. The other 44.8% of the variation was accounted for by factors other than those included in this study. More studies need to be carried out to find out those other factors influencing the levels of glycosylated hemoglobin.

DISCUSSION

The reference ranges obtained from this study for glycosylated hemoglobin differed with the reference ranges that are given in the reagent literatures. According to IFCC the reference ranges for glycosylated hemoglobin are 2.9-4.2 % which applies to both sexes. According to NGSP/DCCT the reference ranges for glycosylated hemoglobin are 4.8-5.9 % which is applies to both sexes. According to JCCLS the reference ranges for glycosylated hemoglobin are 4.3-5.8%, which is applies to both sexes. These are the same reference ranges used today in all the laboratories in Kenya including those in Kiambu County to interpret patient's glycosylated hemoglobin results; each laboratory selects one of the above depending on the standard operating procedure of that particular laboratory. Reference interval derived from a population different to the individual tested, may give a misleading impression of the status of the individual patient (Solberg *et al*., 1989). Use of the reference values currently used today will lead to mismanagement of patients attending the various hospitals of Kiambu County. This is because the lower reference ranges from the literature are higher than the actual value obtained in this study from the residence of Kiambu County. On the other hand, the upper reference ranges from the literature are lower than the actual value obtained in this study from the residence of Kiambu County.

The difference in the values of the reference range given in the literature which was established from people in a different regions like America, United Kingdom and other oversees countries and the values established in this study could be attributed to different operating conditions in the different laboratories, different criteria for selection of healthy subjects, difference in subject preparation and sample collection, different geographical location of the two populations with different temperatures, altitudes, barometric pressures, humidities and time zones. This contributes to difference in life styles including food eaten, physical activities and so difference in the levels of blood glucose, hematological parameters such as the hemoglobin which influences the levels of glycosylated hemoglobin. The difference would also be as a result of genetic difference between the two populations under comparison (Www. Clinlabnavigator.com, 2016). As a result of this it is recommended that each laboratory establishes its own reference ranges for the analytes being analysed in that particular laboratory using samples from the normal population within that geographical region.

Four factors correlated with the dependent variable glycosylated hemoglobin: hemoglobin, packed cell volume, mean cell hemoglobin and random blood glucose. This means that an increase in random blood glucose, hemoglobin, packed cell volume, and mean cell hemoglobin, respectively, increases glycosylated hemoglobin .This means therefore the levels of the above variables need also to be considered when interpreting the levels of the glycosylated haemoglobin

RECOMMENDATIONS:

This study recommends that the established reference values should be circulated to all hospitals in the county of Kiambu and be used henceforth in management of patients with glucose impairment. A similar study to be conducted to establish HbA1c reference values in Kiambu County for children and any other age category not included in the study. A similar study to be conducted for all other biochemical analytes and other disciplines of laboratory science for the whole population in the county. With the references ranges for all analytes for the Kiambu County in place, the dependence of literature reference ranges from other population and regions will be phased out. Further research to be conducted to identify other factors that influence the levels of glycosylated hemoglobin.

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REFERENCES

1. Sidorenkov, G.1., Haaijer-Ruskamp, F.M., de Zeeuw, D. and Denig, P. (2011). A longitudinal study examining adherence to guidelines in diabetes care according to different definitions of adequacy and timeliness. *PLoS One*, 6(9):e24278.
2. Grasbeck, R. and Saris, N.E. (1962). Establishment and use of normal values. *Scandinavian Journal of Clinical Laboratory Investigations*, 26 (Suppl. 110):62-63.
3. Martin, H.F., Gudzinoweiz, B.J. and Fanger, H. (1975). Normal values in Clinical Chemistry. *A guide to statistical analysis of laboratory data*, New-fangled York, Merkel Dekker, Inc.
4. Galen, R.S. (1977). The normal range: a concept in transition. *Archives of Pathology and Laboratory Medicine,* 101(11):561-565.
5. Solberg, H.E. and Grasbeck, R. (1989). Reference values. *Advanced Clinical Chemistry*, 27:1-79.
6. http://www.Clinlab navigator.com/reference range.html [accessed on 29th January 2017].

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