

## RELATIONSHIP BETWEEN ESTIMATED AVERAGE GLUCOSE AND GLYCATED HEMOGLOBIN

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### ABSTRACT

**Objective:** To correlate estimated average glucose (eAG) with glycated hemoglobin (HbA1c) among patients referred from medical and surgical OPDs.

**Methodology:** This cross-sectional study was carried out between January 2016 to July 2017 at the Department of Pathology and Medicine, PNS HAFEEZ Hospital, in collaboration with Department of Chemical Pathology, AFIP. A total of 110 males and 122 females were finally selected for inclusion in the study. They were sampled for fasting plasma glucose, HbA1c and insulin levels. Estimated average glucose (eAG) was calculated as per A1c derived average glucose (ADAG) formula; while insulin resistance was calculated as per the recommendations of Matthew's et al.

**Results:** Pearson's correlation measured between eAG with age, fasting plasma glucose (FPG) and HbA1c were as: [Age:  $r = 0.032$  ( $p = 0.629$ )], [FPG:  $r = 0.547$  ( $p < 0.001$ )], [HOMA-IR:  $r = 0.259$  ( $p < 0.001$ )]. The comparison of various groups based upon FPG results, eAG derived from ADAG equation depicted an increasing trend from group-1 to group-4 as: Group-1 ( $n = 117$ ): [6.17 (95% CI: 5.92-6.42)], to group-2 ( $n = 45$ ): [6.36 (95% CI: 6.02-6.70)], to group-3 ( $n = 43$ ): [6.79 (95% CI: 6.41-7.18)], to group-4 ( $n = 23$ ): [8.66 (95% CI: 7.61-9.70)], ( $p < 0.001$ ).

**Conclusion:** Estimated average glucose (eAG) utilizing HbA1c demonstrated significant positive correlation with fasting plasma glucose.

**Key Words:** Estimated average glucose, Glycated hemoglobin, Fasting plasma glucose, HbA1c

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### INTRODUCTION

Though human species had for centuries been fighting various nutritional deficiency disorders and infections; diabetes mellitus has emerged as the current day plague of today's civilization where not dearth rather exuberance in food supplies has appeared as the major source of morbidity and mortality<sup>1</sup>. Though the riches and developed societies had been facing this disease of metabolism for some time, the recent diabetes pandemic is fast challenging the emerging economies and developed worlds. Unlike the therapeutics side of the disease where multiple options seem to be now available in physician's hand, the diagnosticians still remain dependent upon blood glucose based modes of testing including fasting plasma glucose, oral glucose tolerance, post-prandial glucose and continuous glucose monitoring.

Glycated hemoglobin (also termed as HbA1c and sometimes just A1c) has been introduced lately to

the physician's arsenal to monitor the diabetic control among patients. However, the American Diabetic Association (ADA) has now recommended extension of its role from monitoring diabetic control to diagnostic use<sup>2</sup>. Although this new dimension to HbA1c has broadened its clinical utility, but the clinician in office and patients at large still try to correlate the 2 measures i.e., HbA1c and plasma glucose together to have a simplified version of understanding regarding diabetes both in terms of diagnosis and management. Nathan et al<sup>3</sup> in this regard has suggested a mathematical equation to provide an eAG (estimated average glucose) which relies upon the HbA1c results, which is currently being recommended for both patients with or without diabetes, types of diabetes mellitus and across all races and ethnicities. Moreover, various endorsements of this mathematical HbA1c models for predicting patient's average blood glucose have also been received by International Diabetic Federation (IDF), American Association of Clinical Chemistry (AACC), International Federation of Clinical Chemistry (IFCC) and European

Association for Study of Diabetes (EASD)<sup>4</sup>. Contrary to the promise and the over-simplicity which eAG provides in clinics, counter-narratives to its use have also emerged<sup>5</sup>. Literature review also suggests that the role of other factors like age, gender, race and genetics are important in the buildup of HbA1c. Bergenstal et al<sup>6</sup> have demonstrated black subjects to have higher A1c values than white subjects at levels of plasma glucose lower than white population. This dimension of A1c-glucose axis becomes even more significant as the people from Asians and specifically from sub-continental region have been suggested to have a more diabetogenic phenotype and varying hemoglobin glycation rates in comparison to their Caucasian counterparts from where most data has evolved<sup>7,8</sup>.

Finally the role of genetics can't be undermined where it is believed that population differences due to epigenetic changes can have affect on various enzymatic pathways linking hemoglobin glycation rates, deglycation by amadoriases and factors like glycation gaps(GG)<sup>9-11</sup>. Furthermore, we had to realize the sub-continental phenotype in broader physiological clarity where anemia causing lower hemoglobin levels, a society with growing urbanization trends and endemic vitamin deficiencies thus could present a different or an alternative picture for this A1c-glucose link<sup>1,12,13</sup>. Though some aspects of glycated hemoglobin have been discussed on pakmedinet.com but still the authors were not able to find studies discussing HbA1c results associated with eAG in our set up.

Keeping in view the non-availability of local research on this subject and probable differences in regional prevalence of diabetes mellitus, we decided to correlate our HbA1c results with A1c derived average glucose (ADAG) equation providing eAG for its further application in our set up.

## METHODOLOGY

This cross-sectional study was carried out between January 2016 to July 2017 at the Department of Pathology and Medicine, PNS HAFEEZ Hospital, in collaboration with Department of Chemical Pathology, AFIP. The target population was subjects who were referred from medical and surgical OPDs for evaluation of their fasting plasma glucose in exact medical fasting status. These subjects were briefed regarding participation into the study, study objectives, ethical considerations and purpose of research followed by signing a written consent Performa.

Non-probability convenience sampling strategy was employed to select individuals for sampling. History and clinical examination was carried out along with recording of blood pressure and various anthropometric indices. Subjects who had some chronic ailment, diabe-

tes mellitus on medication, using some other medication which can alter glucose measurements, pregnancy or having an inappropriate medical fasting status were excluded from the study. Finally selected (n=232) subjects were enrolled and sampled for blood.

10 ml of blood was drawn into plain tubes, ethylene diamine tetra acetic acid (EDTA) bottles and sodium fluoride bottles for estimation of fasting plasma glucose (FPG), serum insulin and HbA1c. 04 samples for insulin and glycated hemoglobin were not included due to hemolysis or quantity was not sufficient. Glucose was analyzed by GOD-PAP method. Glycated hemoglobin was measured by fast ion-exchange resin separation method. Serum insulin was measured using chemiluminescence's technique on Immulite® 1000. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as per the method of Mathew's et al<sup>14</sup>. Keeping in view of the total E-8 sector population around 10000, 95% confidence interval and with 20% population proportion, sample size was estimated to be around 240. Estimated Average Glucose (eAG) was derived from ADAG equation as per Nathan et al<sup>3</sup>: estimated average glucose (eAG) in mmol/L = 1.583 (HbA1c-2.52).

All data were entered into Excel program (Microsoft office-2007) and later moved into SPSS version-15. Descriptive statistics in terms of mean  $\pm$ SD intervals were calculated for age. Parameters including age, fasting plasma glucose, HbA1c and HOMA-IR were evaluated for differences in gender by utilizing t-statistics. Pearson's correlation was calculated for age, eAG, fasting plasma glucose, HOMA-IR and HbA1c. Then the results of fasting plasma glucose were grouped into 4 categories as Group-1 (fasting plasma glucose <5.1 mmol/L), Group-2 (fasting plasma glucose 5.1-5.5 mmol/L), Group-3 (fasting plasma glucose 5.6-6.9 mmol/L) and Group-4 (fasting plasma glucose >6.9 mmol/L). Results of eAG, HOMA-IR and HbA1c were analyzed for differences between the above groups by one way ANOVA. A p value of <0.05 was kept as significant.

## RESULTS

Our sample had 110 males (mean age =47.98  $\pm$ 11.30 years) and 122 females (Mean age =45.27  $\pm$ 12.42 years). Glycated hemoglobin and eAG as per ADAG formula (mmol/L) showed slightly higher results among females as depicted in Table 1. eAG demonstrated maximum correlation with fasting plasma glucose as demonstrated in Table 2.

The differences between HbA1c% across various groups formulated on the basis of fasting plasma glucose demonstrated a periodic worsening from normoglycemia to hyperglycemia as depicted in figure 1. Figure 2 shows a gradual worsening of insulin resistance as measured by HOMA-IR from group-1 to group 4. The

**Table 1: Gender differences for age, HbA1c, HOMAIR, eAG and fasting plasma glucose**

Variables	Gender	Frequency	Mean	SD	P value
Age (years)	Male	110	47.98	11.30	0.085
	Female	122	45.27	12.42	
HbA1c (%)	Male	108	5.60	1.01	0.017
	Female	120	5.91	0.93	
HOMA-IR	Male	108	2.47	2.80	0.188
	Female	120	3.14	4.60	
eAG as per ADAG Criteria (mmol/L)	Male	108	6.34	1.60	0.041*
	Female	120	6.78	1.61	
Fasting Plasma Glucose (mmol/L)	Male	110	5.86	2.61	0.127
	Female	122	5.41	1.87	

\*Data analyzed by independent sample t-statistics

**Table 2: Pearson’s correlation as measured between eAG with age, fasting plasma glucose and HbA1c**

Parameters	Pearson’s Correlation	Age (years)	Fasting Plasma Glucose (mmol/L)	HOMA-IR	HbA1c (%)	eAG (ADAG Criteria) (mmol/L)
Age (years)	Pearson’s Correlation	1	-0.013	0.052	0.028	0.032
	Sig. (2-tailed)		0.847	0.431	0.669	0.629
	n	232	232	228	228	228
Fasting Plasma Glucose (mmol/L)	Pearson’s Correlation	-0.013	1	0.466**	0.568**	0.547**
	Sig. (2-tailed)	0.847		0.000	0.000	0.000
	n	232	232	228	228	228
HOMA-IR	Pearson’s Correlation	0.052	0.466**	1	0.282**	0.259**
	Sig. (2-tailed)	0.431	0.000		0.000	0.000
	n	228	228	228	224	224
HbA1c (%)	Pearson’s Correlation	0.028	0.568**	0.282**	1	0.970**
	Sig. (2-tailed)	0.669	0.000	0.000		0.000
	n	228	228	224	228	228
eAG (ADAG) Criteria (mmol/L)	Pearson’s Correlation	0.032	0.547**	0.259**	0.970**	1
	Sig. (2-tailed)	0	.629	0.000	0.000	
	n	228	228	224	228	228

\*\* Correlation is significant at the 0.01 level (2-tailed)

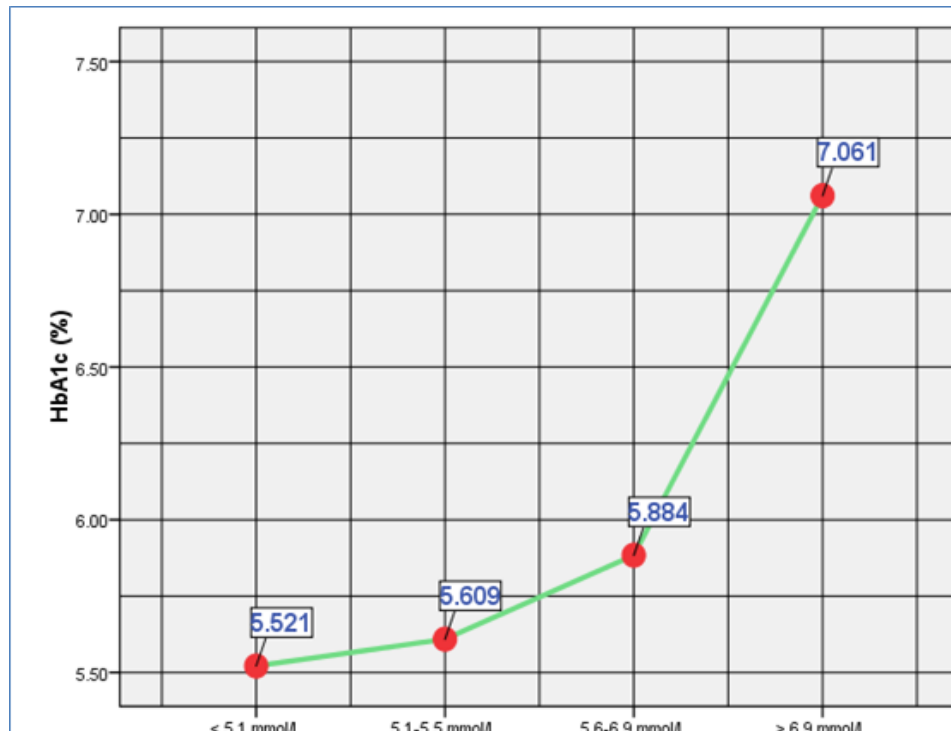
comparison of various groups based upon FPG results, eAG (ADAG equation) depicted an increasing trend from group-1 to group-4 as shown in Figure 3.

## DISCUSSION

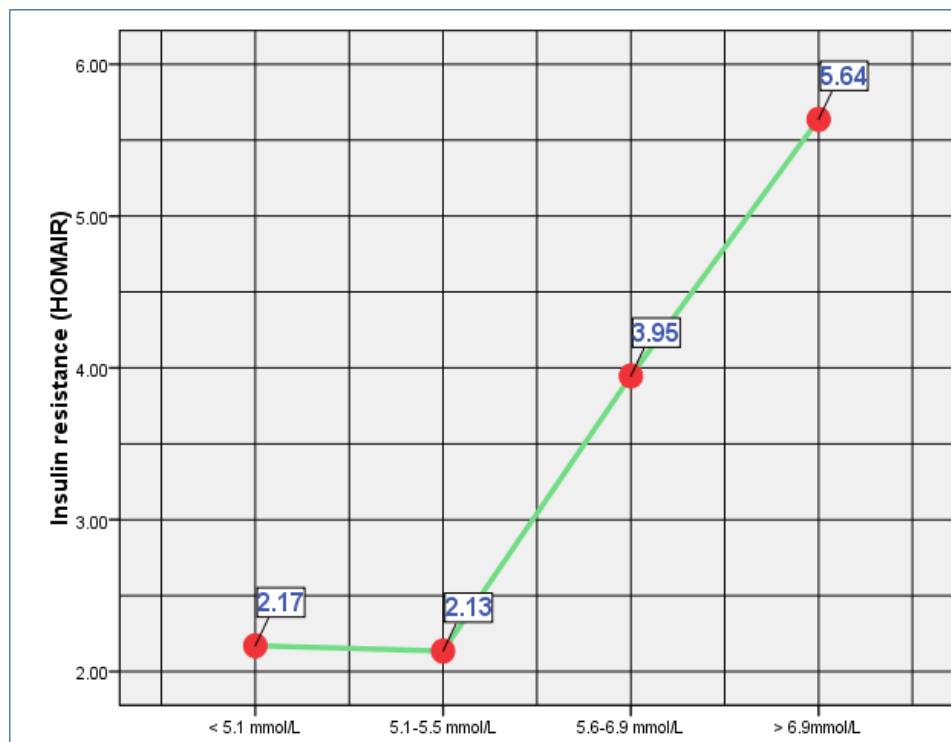
Our study demonstrated that the results of HbA1c derived ADAG measures i.e. eAG showed a significant correlation with fasting plasma glucose and this measure also showed a slight degree of significant positive correlation with insulin resistance. Kim et al<sup>15</sup> and Bozkaya et al<sup>16</sup> have shown correlations coefficients from 0.672 and 0.757 for eAG with FPG which are close but

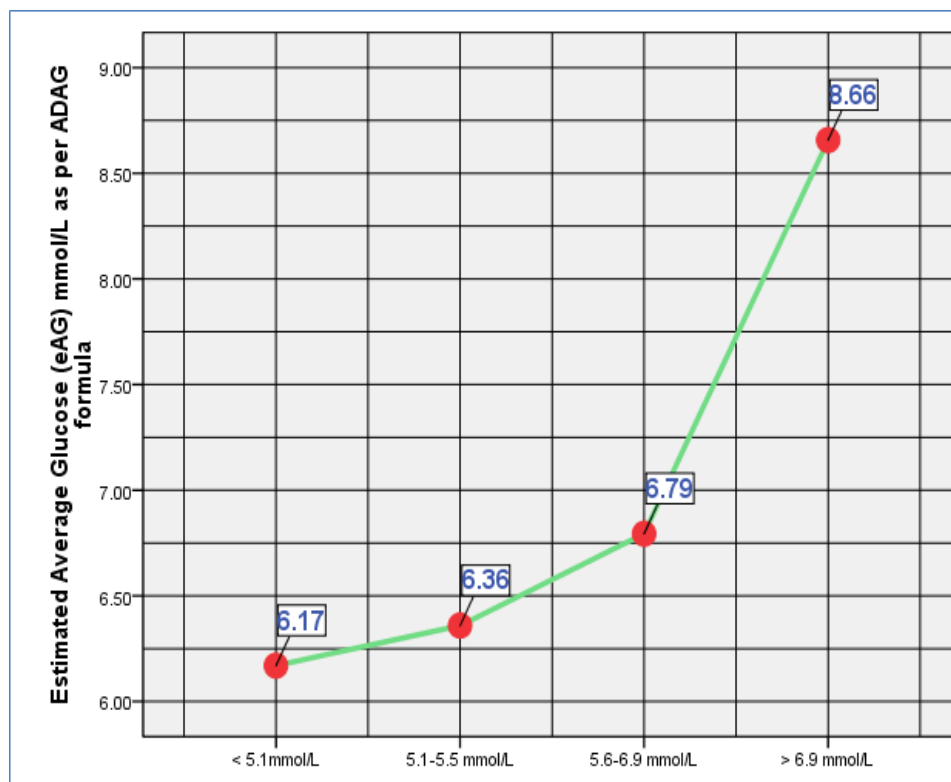
slightly higher than our results. Our data shows that these calculated eAG results probably signifies the relationship between glucose-A1c axis. However, researchers like Hempe et al<sup>17</sup> and Chalew et al<sup>18</sup> have not shown eAG to be a worthwhile clinical marker for clinical use. These contrasts in some studies can be explained as: firstly, these studies have utilized self-monitored mean blood glucose (SMBG) rather than FPG as it is believed that average of daily peaks and troughs could be more variable as well as instrument and haemoconcentration dependent; while FPG can represent a sum of uniform biological behavior over a prolonged duration of time

**Figure 1: Differences in HbA1c (%) among formulated groups based upon fasting plasma glucose (P <0.001)**



**Figure 2: Differences in insulin resistance calculated as per HOMA-IR index among formulated groups based upon fasting plasma glucose (P <0.001)**



**Figure 3: Differences in eAG among formulated groups based upon fasting plasma glucose (P <0.001)**

like 8-12 hours. So in comparison to SMBG the results of FPG can be predicted to be less variable and thus more representative of eAG. Secondly, Hempe et al<sup>17</sup> have segregated subjects based upon hemoglobin glycation index by subtracting actual A1c results from predicted A1c results utilizing the method of Soros et al<sup>19</sup>. However, subjects in our study were not segregated based upon hemoglobin glycation index.

A study carried out at Shenzhen (China) has shown higher HbA1c levels among males; our study has rather demonstrated opposite results with higher HbA1c and thus higher eAG among female population<sup>20</sup>. With a sample size running in thousands the findings by Ma et al<sup>21</sup> seems significant but this regional study may be inclusive of females with iron deficiency anemia which can attribute to these contrasts observed in our results. Similar findings have also been observed in other studies<sup>22,23</sup>. This finding is pertinent for diabetologists and specialists interpreting lab data of HbA1c as associated iron deficiency anemia may result in false positive diagnosis of diabetes mellitus<sup>23,24</sup>.

### LIMITATIONS

Certain limitations to our findings must be acknowledged. Firstly, it is to be acknowledged that the methodological differences have been highlighted to cause variation in glycated hemoglobin<sup>25</sup>. We have utilized the

most cost-effective and routinely in vogue testing methodology i.e., fast ion-exchange resin separation method which may have caused some more variation. Secondly, it's a cross-sectional study and other researchers are encouraged to do further research to conclude the variation of this equation between methodologies to avoid any inter-lab differences in reporting eAG reporting.

### CONCLUSION

Estimated average glucose (eAG) utilizing HbA1c demonstrated significant positive correlation with fasting plasma glucose. Moreover, eAG was also shown to be positively correlated with insulin resistance. However, caution must be exercised in interpreting data in patients with associated iron deficiency anemia which is quite prevalent in third world countries.

### CLINICAL IMPLICATIONS

Our study has following clinical implications: Provided that IDG, IFCC and EASD had strong recommendations to incorporate eAG measures to be reported with HbA1c results, most regional labs in our region have yet not incorporated such a measure which allows both clinicians and patients to have fairly better understanding about interpreting HbA1c results. The study also remains instrumental in identifying the glucose-A1c axis in the Asians as our population seems to be more dia-

betes prone even at a lower fasting plasma glucose result. Utilizing such an equation on our population with lesser literacy rate will indeed pave way for improved diabetes control outcomes.

## REFERENCES

1. Unnikrishnan R, Pradeepa R, Joshi SR, Mohan V. Type 2 Diabetes: Demystifying the Global Epidemic. *Diabetes* 2017; 66:1432-42.
2. Handelsman Y, Bloomgarden ZT, Grunberger G, Umpierrez G, Zimmerman RS, Bailey TS et al. American association of clinical endocrinologists and american college of endocrinology-clinical practice guidelines for developing a diabetes mellitus comprehensive care plan-2015. *Endocr Pract* 2015; 21:413-37.
3. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care* 2008; 31:1473-8.
4. Hanas R, John G. 2010 consensus statement on the worldwide standardization of the hemoglobin A1C measurement. *Diabetes Care* 2010; 33:1903-4.
5. Young IS. Counterpoint: The reporting of estimated glucose with hemoglobin A1c. *Clin Chem* 2010; 56:547-9.
6. Bergenstal RM, Gal RL, Connor CG, Gubitosi-Klug R, Kruger D, Olson BA et al. Racial differences in the relationship of glucose concentrations and hemoglobin A1c levels. *Ann Intern Med* 2017; 167:95-102.
7. Patel SA, Shivashankar R, Ali MK, Anjana RM, Deepa M, Kapoor D et al. Is the "South Asian Phenotype" Unique to South Asians?: Comparing cardiometabolic risk factors in the CARRS and NHANES Studies. *Glob Heart* 2016; 11:89-96.
8. Abate N, Chandalia M. Risk of obesity-related cardiometabolic complications in special populations: a crisis in Asians. *Gastroenterology* 2017; 152:1647-55.
9. Merino-Torres JF, Fajardo-Montañana C, Ferrer-García JC, Piñón-Sellés F. Hemoglobin Glycosylation Index is not related with blood glucose. *J Diabetes Complications* 2003; 17:249-53.
10. Brown SM, Smith DM, Alt N, Thorpe SR, Baynes JW. Tissue-specific variation in glycation of proteins in diabetes: evidence for a functional role of amadoriase enzymes. *Ann N Y Acad Sci* 2005; 1043:817-23.
11. Cohen RM, Snieder H, Lindsell CJ, Beyan H, Hawa MI, Blinko S et al. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins. *Diabetes Care* 2006; 29:1739-43.
12. Unnikrishnan R, Mohan V. Challenges in estimation of glycated hemoglobin in India. *Diabetes Technol Ther* 2013; 15:897-9.
13. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. *Iran Biomed J* 2014; 18:88-93.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28:412-9.
15. Kim HY, Lee SY, Suh S, Kim JH, Lee MK, Park HD. The relationship between estimated average glucose and fasting plasma glucose. *Clin Chem Lab Med* 2013; 51:2195-200.
16. Bozkaya G, Ozgu E, Karaca B. The association between estimated average glucose levels and fasting plasma glucose levels. *Clinics (Sao Paulo)* 2010; 65:1077-80.
17. Hempe JM, Soros AA, Chalew SA. Estimated average glucose and self-monitored mean blood glucose are discordant estimates of glycemic control. *Diabetes Care* 2010; 33:1449-51.
18. Chalew SA, Hempe JM, McCarter R. Clinically significant disagreement between mean blood glucose and estimated average glucose in two populations: implications for diabetes management. *J Diabetes Sci Technol* 2009; 3:1128-35.
19. Soros AA, Chalew SA, McCarter RJ, Shepard R, Hempe JM. Hemoglobin glycation index: a robust measure of hemoglobin A1c bias in pediatric type 1 diabetes patients. *Pediatr Diabetes* 2010; 11:455-61.
20. Ma Q, Liu H, Xiang G, Shan W, Xing W. Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus. *Biomed Rep* 2016; 4:737-40.
21. Akhtar S, Ahmed A, Ahmad A, Ali Z, Riaz M, Ismail T. Iron status of the Pakistani population-current issues and strategies. *Asia Pac J Clin Nutr* 2013; 22:340-7.
22. Rajagopal L, Ganapathy S, Arunachalam S, Raja V, Ramraj B. Does Iron deficiency anaemia and its severity influence HbA1C level in non diabetics? an analysis of 150 cases. *J Clin Diagn Res* 2017; 11:EC13-5.

23. Madhu SV, Raj A, Gupta S, Giri S, Rusia U. Effect of iron deficiency anemia and iron supplementation on HbA1c levels-implications for diagnosis of pre-diabetes and diabetes mellitus in Asian Indians. *Clin Chim Acta* 2017; 468:225-9.
24. Coban E1, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol* 2004; 112:126-8.
25. Yun YM, Ji M, Ko DH, Chun S, Kwon GC, Lee K et al.

Hb variants in Korea: effect on HbA1c using five routine methods. *Clin Chem Lab Med* 2017;55:1234-42.

#### CONTRIBUTORS

SHK conceived the idea, planned the study, and drafted the manuscript. NA, AASG, NF, AI, SMM and MY helped acquisition of data and did statistical analysis. NSKN critically revised the manuscript and supervised the study. All authors contributed significantly to the submitted manuscript.