The Professional Medical Journal
www.theprofesional.com

https://doi.org/10.29309/TPMJ/2021.28.08.6121

ORIGINAL PROF-0-6121

Effect of anemia on HbA1c level in subjects with normal glucose tolerance.

1. M.Phil Student Biochemistry
Baqai Medical University Karachi.
2. Ph.D
Professor & Chairman Biochemistry
Baqai Medical University Karachi.
3. Ph.D
Professor & Head Biochemistry
Baqai Medical University Karachi.
4. M.Phil
Research Officer
Baqai Institute of Diabetology and Endocrinology Karachi.
5. Ph.D
Assistant Professor Biochemistry
Baqai Medical University Karachi.
6. M.Phil, Ph.D
Assistant Professor Physiology
Baqai Medical University Karachi.
7. FRCP
Professor Medicine
Baqai Institute of Diabetology and Endocrinology Karachi.

Correspondence Address:
Dr. Asher Fawwad
Department of the Biochemistry
Baqai Medical University Karachi.
asherfawwad@baqai.edu.pk

Article received on:
01/10/2020
Accepted for publication:
21/12/2020

Muhammad Saeed[1], Iftikhar Ahmed Siddiqui[2], Asher Fawwad[3], Anum Butt[4], Kahkashan Perveen[5], Ruqaya Nangrejo[6], Abdul Basit[7]

ABSTRACT… Objective: To evaluate the effect of iron deficiency anemia (IDA) on HbA1c levels in non-diabetic Pakistani individuals. **Study Design:** Observational Study. **Setting:** Baqai Institute of Diabetology and Endocrinology, Baqai Medical University Karachi. **Period:** March 2019 to May 2019. **Material & Methods:** After approval by the ethics committee of BMU. A world Health Organization (WHO) criterion was used for screening normal glucose tolerance. Subject with type 1 and 2 diabetes, and gestational diabetes were excluded. Selected subjects were categorized into 2 groups (anemic and non-anemic). Data was collected on a structured questionnaire. **Result:** Out of 139 subjects, 72 were males and 67, were females. Anemia was more common in males as compared to females (34.7% vs 19.4%). Among males, HbA1c level was found higher in anemic subjects with MCV<76fl than anemic subjects with MCV>76fl and subjects without anemia. While in females, HbA1c level was similar in both anemic and non-anemic subjects. **Conclusion:** HbA1c as a diagnostic marker should be assessed carefully as the presence of IDA can lead to falsely elevated HbA1c levels in non-diabetic subjects.

Key words: Diabetes, Hemoglobin, Iron Deficiency Anemia.

Article Citation: Saeed M, Siddiqui IA, Fawwad A, Butt A, Perveen K, Nangrejo R, Basit A. Effect of anemia on HbA1c level in subjects with normal glucose tolerance. Professional Med J 2021; 28(8):1172-1177. https://doi.org/10.29309/TPMJ/2021.28.08.6121

INTRODUCTION

Globally, Anemia is major health problem; about 1.62 billion people (24. 8%) are affected by Anemia.[1] The prevalence of anemia is 43% in developing countries whereas 9% in developed nations.[2] All age groups, more commonly preschool-aged children and pregnant women are affected by anemia.[3] Anemia, an unrecognized complication of diabetes mellitus contributes to the pathogenesis and progression of various problems.[4] The most common cause of anemia is Iron deficiency anemia (IDA).[5] Worldwide around two billion population suffers from anemia from which 50% have IDA.[6,7]

Firstly, it was assumed that HbA1C was altered only by blood glucose levels.[8] Although many studies reported, various other confounding factors affects HbA1C levels, beside with diabetes mellitus (DM), specially IDA. Christy and his colleagues evaluated that for last three months maintained plasma glucose levels contributing to controlled level of HbA1C (< 6.5%).[9]

Multiple studies had been conducted out to investigate consequence on HbA1c levels of Iron Deficiency Anemia in patient with diabetes and non-diabetic individuals, while some of the studies was conducted for comparing HbA1c variation in both these groups.[10] The US National Health and Nutrition Survey found IDA has been linked with alterations in HbA1c ranges from <5.0 to ≥5.5% and strongly rises in the patients of absolute HbA1c levels 2 months after treatment of anemia.[11,12] Similarly, Chinese Health and Nutrition Survey reported iron deficiency alone or IDA had an increased in subjects with prediabetes using HbA1c alone when compared with using both HbA1c and fasting blood glucose as the diagnostic criteria.[13]

Alap L .,et al 2014, reported, higher HbA1c levels in IDA subjects was reduced by following iron therapy.[14] Another study, iron deficiency elevated the HbA1c levels in diabetic subjects with iron-sufficient individuals harmonized for fasting plasma glucose levels.[15] On the other hand study on anemia and non-anemia diabetic participants with as well as without iron deficiency shown no alterations in the association between fasting glucose and HbA1c when examined individually.[16] Due to the changes in the results of various researches, we interested to explore the effects of Iron Deficiency Anemia on HbA1c levels in non-diabetics between anemic and non-anemic subjects.

MATERIAL & METHODS

The observational study was carried out in BIDE, Baqai Medical University (BMU) Karachi, Pakistan, after approved by the ethics committee of BMU (Ref: BMU-EC/2018-03). Total 139 subjects out of which 72 males and 67 females were included in this study. In this study (anemic and non-anemic) non-diabetic subject was included. Type 1 and 2 diabetes, gestational diabetes, renal disease, any medications, effecting HbA1c/Iron level, Alcoholism (on history base), Known case of hypo and hyperthyroidism were excluded from the study. The selected participants visited the hospital at least 8 hours of overnight fasting. According to WHO measures each participant was screened, either the fasting plasma glucose (FPG) or the 2-hours plasma glucose (2-h PG) value during a 75g oral glucose tolerance test (OGTT).[19] A written consent agreement was obtained from each participant and data was collected by means of a questionnaire.

By using sterilized disposable vacutainer tubes containing EDTA K2 (for haemoglobin A1C; HbA1c), gel (for lipids) and sodium fluoride (for glucose) blood samples were collected. Complete blood count, HbA1c (%), Hb electrophoresis, Serum Iron /TIBC, Ferritin were also performed by using standard method. Plasma glucose glucose oxidase peroxidase, total cholesterol cholesterol oxidase phenol 4-amino antipyrine peroxidase (CHOD-PAP), glycerolphosphate oxidase-P-aminophenzone (GPOPA) was obtained by triglycerides. Enzymatic calorimetry, high-performance liquid chromatography by low-density lipoprotein cholesterol (LDL-C) by CHOD-PAP, and HbA1c.[17] CBCs were performed using an automated hematology analyzer (MEK-6450). Celltac α Nihon Kohden).[18] The results of the plasma glucose test were as follows: Isolated IFG was defined as the daily fasting plasma glucose level from 110 mg / dL to 125 mg / dL with PGL -140 mg / dL. dL.[19] Using the World Health Organization definition of anemia, there are different restrictions for men and women; High hemoglobin (-16 g / dl in men and -15 g / dl in women), common hemoglobin (-13 g / dl in men and -12 g / dl in women).[20]

Statistical Package for Social Sciences (SPSS) Version 20 was used to perform statistical analysis. While categorical variables were presented as n (%) whereas continuous variables were presented as mean ±SD.

RESULT

Table-I shows that in male group, anemic subjects with MCV<76fL had strong family history of diabetes and higher cholesterol and random blood sugar level as compared to anemic subjects with MCV>76fL and subjects without anemia. Figure-1 reveals that mean HbA1c level was also found higher in anemic subjects with MCV<76fl than anemic subjects with MCV>76fl and subjects without anemia.

Table-II shows that in female group, family history of diabetes and blood sugar levels were almost similar among all subjects. HbA1c level was also found similar in anemic and non-anemic females (Figure-2).

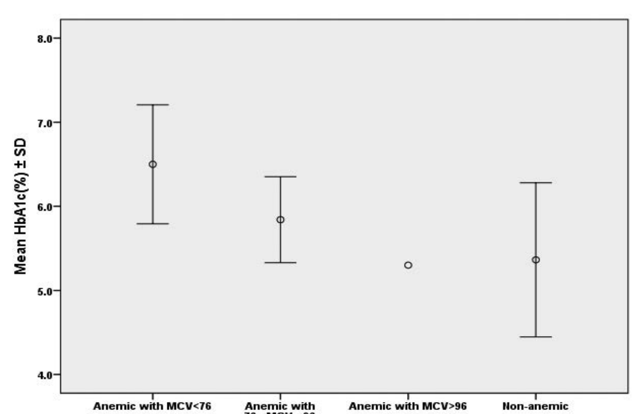

Figure-1. HbA1c level according to anemia in males.

Variable	Anemia			Non-anemia
	MCV<76	76≤MCV≤96	MCV>96	
n(%)	2(2.8%)	22(30.5%)	1(1.4%)	47(65.3%)
Age (years)	35±0	37.24±13.19	26±0	40.3±13.15
BMI (kg/m^2)	28.15±2.73	26.17±3.9	22.14±0	25.67±3.22
Marital status				
Single	0(0%)	2(9.1%)	1(100%)	10(21.3%)
Married	2(100%)	20(90.9%)	0(0%)	37(78.7%)
Blood Pressure				
Systolic BP	120±0	109.44±16.97	110±0	111.49±12.3
Diastolic BP	80±0	72.22±10.6	80±0	74.46±7.71
Tobacco				
No	2(100%)	17(77.3%)	1(100%)	43(91.5%)
Yes	0(0%)	5(22.7%)	0(0%)	4(8.5%)
Smoking				
No	2(100%)	19(86.4%)	1(100%)	44(93.6%)
Yes	0(0%)	3(13.6%)	0(0%)	3(6.4%)
Alcohol				
No	2(100%)	22(100%)	1(100%)	46(100%)
Yes	0(0%)	0(0%)	0(0%)	0(0%)
Dyslipidemia				
No	1(50%)	4(18.2%)	0(0%)	17(36.2%)
Yes	1(50%)	18(81.8%)	1(100%)	30(63.8%)
Hypertension				
No	2(100%)	19(86.4%)	1(100%)	39(83%)
Yes	0(0%)	3(13.6%)	0(0%)	8(17%)
Stroke				
No	2(100%)	21(95.5%)	1(100%)	47(100%)
Yes	0(0%)	1(100%)	0(0%)	0(0%)
Family history of DM				
No	0(0%)	13(59.1%)	1(100%)	28(59.6%)
Yes	2(100%)	9(40.9%)	0(0%)	19(40.4%)

Table-I. Prevalence of different variables in Anemia and non-anemia Male Subjects.

Variables	Anemia		Non-Anemia
	MCV<76	76≤MCV≤96	
n(%)	6(8.9%)	7(10.5%)	54(80.6%)
Age (years)	36.17±10.52	31.14±8.57	36.6±12.45
BMI (kg/m^2)	28.07±9.71	27.5±6.22	26.49±5.92
Marital Status			
Single	2(33.3%)	3(42.9%)	11(20.4%)
Married	4(66.7%)	4(57.1%)	43(79.6%)
Blood Pressure			
Systolic BP	112±13.04	105.71±12.72	108.75±18.56
Diastolic BP	72±8.37	68.57±9	71.84±9.7
Tobacco			
No	6(100%)	7(100%)	52(96.3%)
Yes	0(0%)	0(0%)	2(3.7%)
Smoking			
No	6(100%)	7(100%)	53(100%)
Yes	0(0%)	0(0%)	0(0%)
Alcohol			
No	6(100%)	7(100%)	54(100%)
Yes	0(0%)	0(0%)	0(0%)
Dyslipidemia			
No	1(16.7%)	1(14.3%)	27(50%)
Yes	5(83.3%)	6(85.7%)	27(50%)
Hypertension			
No	6(100%)	7(100%)	48(88.9%)
Yes	0(0%)	0(0%)	6(11.1%)
Stroke			
No	6(100%)	7(100%)	54(100%)
Yes	0(0%)	0(0%)	0(0%)
Family History of DM			
No	3(50%)	5(71.4%)	29(53.7%)
Yes	3(50%)	2(28.6%)	25(46.3%)

Table-II. Prevalence of different variables in Anemia and non-anemia Female Subjects.

Parameters		Anemia			Non-Anemia
		MCV<76	76≤MCV≤96	MCV>96	
FBS (mg/dl)	Males	93.5±6.36	92.5±5.64	99±0	90.83±8.08
	Females	90.83±7.17	89.14±4.41		89.28±6
RBS (mg/dl)	Males	134.5±0.71	99.32±20.45	113±0	101.62±19.82
	Females	98.17±20.88	99.14±12.93		104.52±16.31
Cholesterol (mg/dl)	Males	200±0	161.59±36.15	142±0	190.72±44.24
	Females	168.6±38.23	164±29.77		195.14±45.33
Triglyceride (mg/dl)	Males	175±0	142.59±91.21	74±0	184.93±100.55
	Females	138.4±78.98	119.17±88.46		115.9±51.92
HDL (mg/dl)	Males	25±0	27.82±4.25	33±0	29.72±4.52
	Females	33.8±7.98	33.17±6.11		36.45±8.43
LDL (mg/dl)	Males	115±0	100.18±32.22	94±0	124.17±38.09
	Females	112.4±20.01	99.83±27.58		123.79±36.16
VLDL (mg/dl)	Males	35±0	28.52±18.24	14.8±0	36.95±20.13
	Females	27.72±15.76	23.83±17.69		23.18±10.41
Non-HDL-C (mg/dl)	Males	175±0	133.76±33.27	109±0	161±43.53
	Females	134.8±32.27	130.83±26.69		158.69±41.75
HbA1c (%)	Males	6.5±0.71	5.84±0.51	5.3±0	5.36±0.92
	Females	5.6±0.44	5.57±0.5		5.34±0.49
Iron (μg/dl)	Males	70±0	83.45±9.97	75±0	-
	Females	73.33±9.16	81.71±10.59		

Table-II. Comparison of Biochemical parameters between anemia and non-anemia subjects.

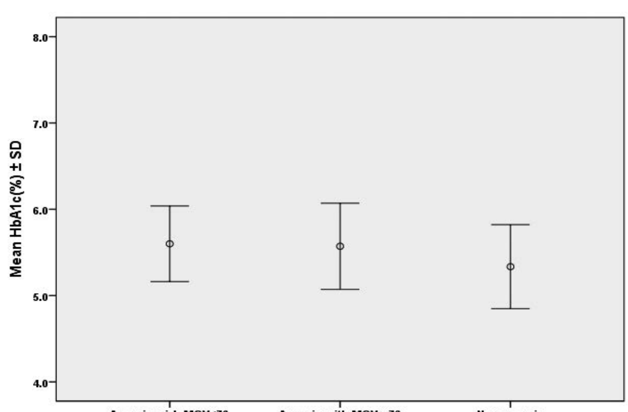

Figure-2. HbA1c level according to anemia in females.

DISCUSSION

Anemia can affect erythropoiesis; RBC production, Hb synthesis, and RBC volume or surface area may decrease. Anemia increases the level of RBC blood circulation, which affects the value of HbA1c. The results of our study indicate that IDA is associated with high concentrations of HbA1c. In addition, iron replacement therapy causes a decrease in HbA1c. Similarly, high levels of HbA1c in iron-deficient adults, diabetes is abnormal after iron replacement.[21] One previous study observed HbA1c concentrations of normal iron deficiency and reduced them to abnormal levels after taking iron supplements.[22] In contrast, there was no change in HbA1c concentration in diabetic patients with diabetes before and after iron therapy.[23] They noted that the reported differences in pre- and post-iron HbA1c concentrations were due to differences in laboratory methods used to measure HbA1c.

Because HbA1c levels predict the risk of many chronic diabetic complications, HbA1c is often used to assess long-term blood glucose control in people with diabetes.[24,25] The results of our study indicate that iron deficiency is associated with high HbA1c levels, which can be a problem in the uncontrolled diagnosis of diabetes in patients with iron deficiency. Iron status should be considered when interpreting HbA1c concentrations in diabetes. Iron replacement therapy is particularly important in patients with iron deficiency, which increases the reliability of HbA1c determination.

A Japanese study of diabetic and non-diabetic pregnant women found a positive correlation between HbA1C and MCV and a negative correlation between red blood cell counts.[26] Similarly, another study from Japan documented a relatively inverse relationship with MCH in assessing pregnant women with conditions such as diabetes.[28] Several studies in diabetics have shown a negative correlation between red blood cell and HbA1C indices: HB, MCV, and MCH.[27,29] Similar results were obtained by Hardikar et al. Diabetes populations were assessed to show an inverse relationship between HbA1c and MCV, MCH, and MCHC.[29]

Anemia was more common in males as compared to females (34.7% vs 19.4%). Among males, 2.8% had anemia with MCV<76fL, 30.5% had anemia with MCV between 76 to 96 fL, 1.4 % had anemia which MCV> 96fL while 65.3% had no anemia. Among females, 8.9% had anemia with MCV<76 fL, and 10.5 % had anemia with MCV between 76 to 96 fL while majority (80.6%) had no anemia.

CONCLUSION

HbA1c as a diagnostic marker should be assessed carefully as the presence of IDA can lead to falsely elevated HbA1c levels in non-diabetic subjects.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Muhammad Saeed	Concept and design, Interpretation of data and wrote the manusript.	
2	Iftikhar Ahmed Siddiqui	Concept and design, edited and approved the manuscript.	
3	Asher Fawwad	Concept and design, edited and approved the manuscript.	
4	Anum Butt	Interpretation of data, Wrote and reviewed the manuscript.	
5	Kahkashan Perveen	Interpretation of data, Write, edited and reviewed the manuscript.	
6	Ruqaya Nangrejo	Literature search, edited and reviewed the manuscript.	
7	Abdul Basit	Concept and design, edited and approved the manuscript.	