Original Article

HbA_{1C} as a Biomarker of Non-alcoholic Fatty Liver Disease: Comparison with Anthropometric Parameters

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Abstract

Background and Aims: Multiple non-invasive methods including radiological, anthropometric and biochemical markers have been reported with variable performance. The present study assessed glycosylated hemoglobin (HbA_{1C}) as a biomarker to predict non-alcoholic fatty liver disease (NAFLD) and its severity, compared with body mass index (BMI), waist to hip ratio (WHR) and waist circumference (WC) ***Methods:*** This case control study included 450 individuals, including 150 cases and 300 age- and gender-matched controls recruited from the Dow Radiology Institute on the basis of radiological findings of fatty infiltration on abdominal ultrasound through convenient sampling. BMI, WHR and WC were measured according to standard protocols. HbA_{1C} was determined by turbidimetric inhibition immunoassay ***Results:*** Among the cases and controls, 66% and 32% had HbA_{1C} levels higher than 5.7% respectively. HbA_{1C} and BMI were significantly associated with NAFLD [crude odds ratio (cOR)=4.12, 2.88, 2.25 (overweight) and 4.32 (obese)]. WC was found to be significantly associated with NAFLD for both genders (cOR in males=5.50 and females=5.79, $p<0.01$). After adjustment for other parameters, HbA_{1C} and WC were found to be significantly associated with NAFLD (aOR=3.40, $p<0.001$) along with WC in males (aOR=2.91, $p<0.05$) and in females (aOR=4.28, $p<0.05$). A significant rise in severity of hepatic steatosis was noted with increases in HbA_{1C}, BMI and WC. HbA_{1C} possessed a positive predictive value of 76% for the study population [0.76, confidence interval (CI): 0.715-0.809], 70.6% for males (0.706, CI: 0.629-0.783) and 80% for females (0.80, CI: 0.741-0.858). ***Conclusions:*** Higher than normal HbA_{1C} and WC measurements possess a more than 70% potential to predict NAFLD. It is the single risk factor that is strongly associated with NAFLD after adjustment for indices of body measurements. HbA_{1C} may be presented as a potential biomarker for NAFLD in examination with other anthropometric measures in the adult population.

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Keywords: NAFLD; HbA_{1C}; Biomarker; Non-diabetic population; Body weight measurements; Body mass index (BMI); Waist circumference (WC).

Abbreviations: aOR, adjusted odds ratio; AUC, area under the ROC curve; BMI, body mass index; CI, confidence interval; cOR, crude odds ratio; DUHS, Dow University of Health Sciences; FLD, fatty liver disease; HbA_{1C}, glycosylated hemoglobin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ROC, receiver operating characteristic; TAG, triacylglycerol; Type II DM, type II diabetes mellitus; WC, waist circumference; WHR, waist to hip ratio.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a condition wherein excess fat accumulates in the liver of people with no history of significant alcohol consumption.[1] Fat molecules are deposited in the form of triacylglycerols (TAGs) in hepatocytes. Hepatic steatosis refers to fatty change in hepatocytes and is largely a benign condition, while, not in a small number of patients, it may trigger an immune response and lead to non-alcoholic steatohepatitis (NASH) followed by cirrhosis and cancer.[2] NAFLD is alarmingly increasing around the globe. The estimated global prevalence of NAFLD ranges from 6.3-33% among the general population, varying among and within populations.[3] The prevalence is highest among obese (57%) and diabetic (90%) populations.[4] A rising trend of prevalence of NAFLD has been observed in line with obesity, at a rate of 25%.[5] Sedentary lifestyle, dyslipidemia and metabolic syndrome are also well documented risk factors for NAFLD,[4] along with other risk factors, such as hepatitis B and C virus infection, Wilson's disease, and chronic blood and kidney diseases.

High blood glucose levels non-enzymatically form glycosylated hemoglobin (HbA_{1C}) as an irreversible reaction. Once formed, HbA_{1C} remains in circulation for 2-3 months; hence, it has been identified as the marker for diabetes diagnosis and control. According to the American Association of Clinical Endocrinologists, an acceptable level of HbA_{1C} in diabetics is <6.5% and reflects good metabolic control;[6] although, tight control is recommended to avoid increased risk of hypoglycemia, but the level of <6.5% is considered as acceptable in this study. Obesity and diabetes have been reported as strong predictors of NAFLD.[7] Therefore, it may be assumed that patients with NAFLD have increased levels of HbA_{1C} as well.

On the contrary, recently emerging data suggest that HbA_{1C} may be raised in the absence of diabetes. Chen *et al*.,[8] in 2020, reported that after multiple adjustments HbA_{1C} serves as a risk factor for NAFLD, with a significant odds ratio of 1.58 in metabolically-intact patients. The South Asian countries have reported a prevalence of 13.9% of NAFLD in the adult healthy population that excludes obesity and diabetes.[9] It is suggested that inter-individual biological differences may also contribute to the elevation of HbA_{1C}, apart from high blood sugar.[10,11]

Similarly, various indices of body measurements, such as body mass index (BMI), waist to hip ratio (WHR), and obesity status, have been linked with insulin resistance, type II diabetes mellitus (Type II DM),[12] and NAFLD. About 20–35% of lean NAFLD cases have been reported from rural areas of some Asian countries.[13] Waist circumference (WC),

on the other hand, has emerged as the physical measure associated significantly with NAFLD.[14] The debate on the relevance of various body weight measurements, including BMI, WHR and WC, has generated much data with conflicting observations regarding their significance as the risk factor for NAFLD.[15,16]

Although liver biopsy is the gold standard for the diagnosis and grading of severity of hepatic steatosis and NASH, due to its invasive nature and cost/risks, alternate non-invasive and cost-effective methods have been widely searched and reported.[17] Radiological diagnoses and grading is widely recommended by nearly all associations for study of liver diseases, such as the European Association for the Study of the Liver, the Italian Association for the Study of the Liver, and the American Association for the Study of Liver Diseases.[18] The non-invasive protocols to diagnose NAFLD cannot equate to the gold standard liver biopsy, but they may help in detecting early steatosis in hepatocytes.

NAFLD is among the most common cause of chronic liver disease.[17] The diagnosis protocol of NAFLD basically addresses pathological/radiological evidence of fatty infiltration, liver biochemistry, and history of alcohol consumption and other chronic diseases. There is dire need of identifying biochemical markers with significant discriminatory performance for NAFLD diagnosis. The present study was, therefore, designed to measure and find the association of HbA_{1C} as a novel biomarker for the diagnosis of NAFLD patients identified through abdominal ultrasound.

Methods

This case control study was conducted at the Dow University of Health Sciences (DUHS). Diagnosis of fatty liver disease (referred to here as FLD) was based on ultrasonographic evidence of fatty infiltration of hepoatocytes.[18] Individuals of both genders in the adult age group, undergoing upper abdominal ultrasonography at the Department of Radiology at the DUHS, were recruited for the study. Those having FLD on ultrasound were identified as cases, while people showing no fatty infiltration were included as controls. Informed consent was obtained after explaining the study procedures and outcomes; those who refused to be included were dropped out. Considering the prevalence of the condition in Pakistan,[6] sample size was calculated by OpenEpi as 104 (52 each in the case and control groups). However, to improve the strength of our study, the total sample size was increased to 450, with case-to-control ratio of 1:2. The participants were recruited through convenient sampling (150 cases and 300 age- and gender-matched controls). The severity of steatosis was graded on the basis of fatty infiltration found on ultrasonography, from grades (1-3) as follows: grade 1 had minimal infiltration, with echogenicity slightly increased; grade 2 had moderate infiltration, with echogenically obscured portal vessel walls; and grade 3 had heavy fatty infiltration.[19]

For the purpose of standardization, subjects undergoing ultrasonography (by two trained sonologists) were included in the study. Patients with chronic liver disease, tumors, acute hepatitis, Wilson's and kidney diseases, known NAFLD, and those having history of alcohol consumption were excluded. History regarding presenting complaints, comorbidities, lifestyle, dietary intake, and medication were recorded on structured proforma. Detailed physical examination was carried out. Height in meters and weight in Kg was recorded for BMI (reported as Kg/m^2). WC and WHR were measured by standard methods.[20] Blood samples were collected in the fasting state, with samples in appropriate bar-coded containers, for estimation of HbA_{1C} by turbidimetric inhibition immunoassay and expressed as percentage (%). The value of 5.7% or below was taken as normal.[21] The study was approved by the institutional review board of DUHS (IRB-447/DUHS/-14) and funded by the Higher Education Commission of Pakistan.

Statistical analysis

Data was analyzed using SPSS version 21.0 and STATA 14. Chi-square, ANOVA and binary logistic regression were used for analysis. Frequencies and proportions were generated for all categorical variables, study participants' characteristics and body weight measurements with NAFLD. These were compared using the chi-square (χ^2) test, while mean differences for anthropometric measures with steatosis severity grades were assessed using ANOVA. Binary logistic regression analyses (univariate and multivariate) were used to assess the factors associated with NAFLD occurrence. Results of regression were reported as crude odds ratio (cOR) and adjusted odds ratio (aOR) with 95% confidence interval (CI). Receiver operating characteristic (ROC) curve was plotted to compare each variable with NAFLD and to find the valid predictive value of HbA_{1C} to diagnose NAFLD. A p value <0.05 was taken as significant.

Results

Baseline characteristics are given in Table 1. Females dominated the sample, with 56% among cases and 60.3% among controls. The mean age of the study sample was 43.96 ± 11.06 years.

Table 2 shows the variations of HbA_{1C}, BMI, WHR, WC and frequency of known diabetics (type II DM) among the various grades of NAFLD. We found that 40% of individuals with type II DM had grade III steatosis, while 23.7% and 10.9% were among grades II and I respectively. Only 7.7% of diabetic people within the study sample did not have FLD.

Odds for HbA_{1C} were significantly high [cOR=4.12 (CI: 2.72-6.25)] and were consistently high after adjusting with history of type II DM and the indices of body measurements [BMI, WHR and WC of 3.40 (CI: 2.19-5.26); in males, 2.08 (CI: 1.06- 4.11) and in females, 5.20 (CI: 2.79-9.68)] (Table 3). BMI was significantly associated with NAFLD; however, after adjustment with type II DM and HbA_{1C}, the odds of BMI were found to retain significance in obese individuals only. Further, after stratification of data on the basis of gender, it became insignificant in males. In both genders, WHR was found to be not significant. Odds for HbA_{1C} and WC were found to consistently be significant in the total study sample as well as in both genders (Table 3).

ROC curve analysis demonstrated a valid positive prediction value for HbA_{1C} in comparison with WC, and HbA_{1C} for a binary outcome (NAFLD) (Fig. 1A, 1B) in both genders. The area under the curve (AUC) was 76% for HbA_{1C} in the overall study population (0.76, CI: 0.715-0.809), being 70.6% for males (0.706, CI: 0.629-0.783) and 80% for females (0.80, CI: 0.741-0.858).

Discussion

Baseline characteristics of the study population are given in Table 1. Age- and gender-matched controls exhibited more than 5.7% HbA_{1C} in 66% and 32% of controls and cases respectively. The other significant presentation was a higher BMI in more than 80% and 70% of controls and cases respectively, which is consistent with others reports.[22,23] Generally, BMI and central obesity are higher in Asian populations.[24] We also confirmed a female preponderance (89.4

Table 1. Baseline characteristics among cases and controls, *n*=450

Variables		Cases	Controls	*p*-value
Age, years	Mean ± SD	44.68 ± 10.62	43.61 ± 11.27	0.333
Gender, *n* (%)				0.378
	Male	66 (44.0)	119 (39.7)	
	Female	84 (56.0)	181 (60.3)	
HbA_{1C}, *n* (%)				<0.001
	≤5.7%	51 (34.0)	204 (68.0)	
	>5.7%	99 (66.0)	96 (32.0)	
Diabetes status, *n* (%)				<0.001
	Yes	29 (19.3)	23 (7.7)	
	No	121 (80.7)	277 (92.3)	
BMI, *n* (%)				<0.001
	Underweight/Normal	18 (12.0)	93 (31.0)	
	Overweight	45 (30.0)	103 (34.3)	
	Obese	87 (58.0)	104 (34.7)	
WHR, *n* (%)				
Male	<0.9	9 (13.6)	47 (39.5)	<0.001
	≥0.9	57 (86.4)	72 (60.5)	
Female	<0.85	16 (19.0)	43 (23.8)	0.391
	≥0.85	68 (81.0)	138 (76.2)	
Waist circumference, *n* (%)				
Male	<90 cm	7 (10.6)	47 (39.5)	<0.001
	≥90 cm	59 (89.4)	72 (60.5)	
Female	<80 cm	3 (3.6)	32 (17.7)	0.002
	≥80 cm	81 (96.4)	149 (82.3)	

vs. 96.4), with higher WC presenting with NAFLD. Attempts are in progress to develop non-invasive methods to predict NAFLD, one being the assessment of HbA_{1C} to detect hepatic steatosis. A cross-sectional study found a significant association of HbA_{1C} with NAFLD in an elderly Chinese population.[25] Similarly, a significant correlation was found between HbA_{1C} and NAFLD in an adult Korean population, again in a cross- sectional setting.[26] However, the cross-sectional design only identifies the prevalence of a factor at a certain point of time. A longitudinal study, on the other hand, suggested that HbA_{1C} may contribute to the development of NAFLD.[8] Chen *et al*.[8] further expressed the need for more studies to test the impact of HbA_{1C} on development of NAFLD.

The present study found a significant association of HbA_{1C} with NAFLD in a case control design, which strengthens the

Table 2. Variations in HbA_{1C} and indices of body measurements with severity of steatosis

	No fatty liver	Fatty liver			*p*-value
		Grade I	Grade II	Grade III	
HbA_{1C} (Mean ± SD)	5.54 ± 0.89	6.21 ± 1.25	6.90 ± 1.85	7.49 ± 2.22	<0.001
BMI (Mean ± SD)	28.38 ± 6.15	29.86 ± 5.92	32.19 ± 5.29	32.36 ± 3.63	<0.001
WHR-Male (Mean ± SD)	0.91 ± 0.11	0.94 ± 0.04	0.96 ± 0.06	0.98 ± 0.09	0.038
WHR-Female (Mean ± SD)	0.90 ± 0.10	0.91 ± 0.07	0.92 ± 0.07	0.95 ± 0.10	0.772
WC-Male (Mean ± SD)	94.16 ± 16.4	101.10 ± 11.0	105.63 ± 14.2	111.2 ± 8.9	<0.001
WC-Female (Mean ± SD)	95.12 ± 14.3	100.38 ± 11.2	101.61 ± 12.2	108.3 ± 6.4	0.003
Diabetes, *n* (%)					<0.001
Yes	23 (7.7)	7 (10.9)	18 (23.7)	4 (40.0)	
No	277 (92.3)	57 (89.1)	58 (76.3)	6 (60.0)	

Table 3. Associations of HbA_{1C}, BMI, history of type II DM, WHR and WC with NAFLD

Variables	cOR (95% CI)	*p*-value	NAFLD		NAFLD-Male		NAFLD-Female	
			aOR (95% CI)	*p*-value	aOR (95% CI)	*p*-value	aOR (95% CI)	*p*-value
HbA_{1C}								
≤ 5.7%	1		1		1		1	
> 5.7%	4.12 (2.72-6.25)	<0.001	3.40 (2.19-5.26)	<0.001	2.08 (1.06-4.11)	0.033	5.20 (2.79-9.68)	<0.001
Diabetes status								
No	1		1		1		1	
Yes	2.88 (1.60-5.19)	<0.001	1.64 (0.86-3.090	0.127	1.08 (0.40-2.86)	0.877	2.21 (0.88-5.52)	0.089
BMI								
Underweight/Normal	1		1		1		1	
Overweight	2.25 (1.22-4.17)	0.009	1.79 (0.94-3.40)	0.074	1.65 (0.66-4.07)	0.278	1.02 (0.36-2.89)	0.962
Obese	4.32 (2.42-7.71)	<0.001	3.30 (1.80-6.050	<0.001	1.73 (0.70-4.26)	0.230	2.90 (1.11-7.58)	0.029
WHR								
Male								
WHR <0.9	1		-	-	1		-	-
WHR ≥0.9	4.13 (1.87-9.13)	<0.001	-	-	2.03 (0.81-5.09)	0.128	-	-
Female								
WHR <0.85	1		-	-	-	-	1	
WHR ≥0.85	1.32 (0.69-2.52)	0.392	-	-	-	-	0.72 (0.32-1.61)	0.432
WC								
Male								
WC <90 cm	1	-	-	-	1	-	-	-
WC ≥90 cm	5.50 (2.31-13.07)	<0.001	-	-	2.91 (1.06-7.94)	0.037	-	-
Female								
WC <80 cm	1	-	-	-	-	-	1	
WC ≥80 cm	5.79 (1.72-19.52)	0.005	-	-	-	-	4.28 (1.10-16.61)	0.035

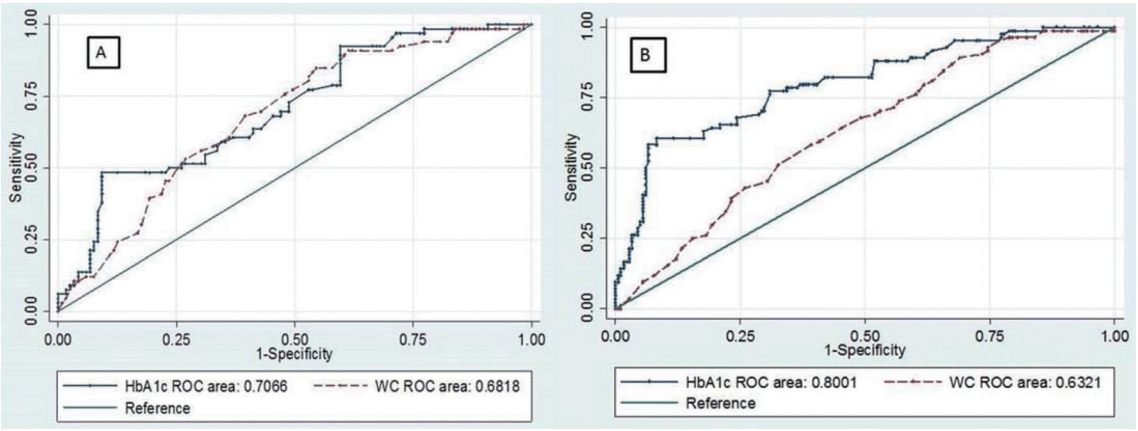

Fig. 1. ROC curve of HbA_{1C} and WC in males (A) and females (B) in relation to NAFLD.

results to be interpreted as a potential predictor of the disease retrospectively. This association was positive both in diabetic, non-diabetic, obese, and lean persons, indicating that those who have HbA_{1C} higher than 5.7% are 4-times more prone to developing fatty liver disease (cOR=4.12, p<0.001). Recently, HbA_{1C} has been reported as risk factor for NAFLD, with an odds ratio of 1.58 (p<0.004).[8] Another study also showed that presence of NAFLD presents a higher risk of glycemic progression and incident diabetes.[27] Therefore, existing data until now suggests that NAFLD and glycemic derangement coexist, but the conflict remains regarding the cause and effect relationship of HbA_{1C} and NAFLD.

Ultrasonography has recently been largely discussed as a useful bedside diagnostic tool to detect hepatic steatosis.[28] The need for non-invasive tools/biomarkers has also become more important, with numerous drug trials underway, some having reached phase IV. Follow-up of these cases is not feasible with multiple biopsies. The ultrasonographic fatty liver indicator is reported to be able to identify mild hepatic steatosis that correlates with histological findings of severity and metabolic parameters.[29] In concordance with this, our results also identified ultrasound as a reliable tool to detect hepatic steatosis and its severity. Ballestri *et al*.[29] also correlated ultrasonographically -detected steatosis with all parameters of glycemic control, except HbA_{1C}.

The present study confirms that the severity grades of steatosis correlate significantly with HbA_{1C} levels as well. Among the body measurements, BMI and WC showed stronger correlation with severity grades of steatosis than WHR in both genders (Table 2). Others have reported that ultrasonographic techniques have been improved over the last decade, but there is still a dire need to develop a combination of pre-test probability based on anthropometric variables and/or biochemical biomarkers with various ultrasonographic techniques[30] which can be applied to the liver biopsy scoring system.[31] Our results confirm that HbA_{1C} and WC along with ultrasonographic evidence of steatosis can detect early fatty change in hepatocytes satisfactorily (Table 2).

HbA_{1C} is produced in direct proportions to the duration and episodes of high blood glucose concentrations.[32] HbA_{1C} may also vary due to biological differences among individuals, apart from hyperglycemic episodes.[33] Hyperglycemic episodes, in addition to the production of advanced glycation end-products also affect lipid metabolism and result in increased synthesis of TAGs that tend to deposit in various tissues of the body, including liver. TAG deposition in adipose tissue increases BMI, while in liver parenchyma it leads to fatty liver. Type II DM has been strongly linked with fatty deposition in liver and HbA_{1C} may be causally associated with NAFLD.[34] On the other hand, obesity in the absence of type II DM also relates to increased fat content in body tissues. Higher BMI has been associated with insulin resistance and increases in HbA_{1C}.[35] However, in addition to this, our study confirms increase in HbA_{1C} levels results in more than 3-times chance of NAFLD development independent of diabetes mellitus.

Studies have demonstrated variable results when comparing effect of age, gender, BMI, and obesity. There are opinions that BMI is not a good indicator of chronic disease association, as compared to abdominal fatness[36] (central obesity, represented by WC). Excess abnormal fat predisposes to obesity-related disease, regardless of total body fat. The present study found both BMI and WC to be significantly different (p<0.001) with presence of NAFLD in both genders (Table 1), while WHR was significantly different only among males. All of these indices were significantly associated with NAFLD (Table 3). However, when it was adjusted for other parameters, this association became weaker, whereas association with WC remained significant both in males and females (aOR 2.91 and 4.28 respectively, p<0.001). This indicates that abdominal obesity is more associated with presence of NAFLD. Even the patients who are lean develop fatty liver if they have central obesity.[37] Both of these conditions are associated with insulin resistance and, hence, high HbA_{1C} may be a common link between NAFLD and type II DM/central obesity.

The results of this study also depicted that, as compared to male patients, females had higher central obesity (Table 1) and NAFLD in concordance to results reported by Dai *et al*.,[38] who also found increased measures of BMI and WC in NAFLD patients. The present study demonstrates that NAFLD can be predicted by a combination of HbA_{1C} and WC both in males (AUC=0.706 and 0.681 respectively) and in females (AUC=0.800 and 0.632 respectively). This is in concordance to others who claimed that a combination of age, sex, WC, alanine aminotransferase, HbA_{1C}, and HOMA-IR with an AUC of 0.87 can best predict NAFLD.[39] With these data, it is tempting to suggest that investigation of HbA_{1C} and central obesity may predict the presence of NAFLD in otherwise healthy individuals.

Conclusions

HbA_{1C} level is significantly associated with presence of NAFLD. Higher than normal HbA_{1C} levels possess greater than 70% potential to predict NAFLD. WC is the second most associated factor with NAFLD. HbA_{1C} is the single risk factor that is strongly associated with NAFLD after adjust-

ment for BMI, WHR and WC. HbA_{1C} may be presented as a novel potential biomarker for NAFLD examined with WC in the adult population.

Limitations

Liver biopsy was not performed owing to its invasive nature, with no justification for the test in controls. Secondly, ultrasonography of liver may not identify cases of NAFLD with early changes; therefore, some of the potential cases may have been grouped as controls.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception and design of the study (MM, ZH), recruitment and examination of the patients (MM), collection of the data (MM), performance of the assays (MM), analysis of the data (MM, ZH), and writing of the paper (MM, ZH).

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