HbA1C as a potential Biomarker of Non-Alcoholic Fatty Liver Disease: comparison with BMI, WHR and WC

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Research article

Keywords: NAFLD, HbA1C, Biomarker, non-diabetic population, Body weight measurements, BMI, WC

Posted Date: August 14th, 2019

DOI: <https://doi.org/10.21203/rs.2.12789/v1>

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is a slow progressing disease common in obese and diabetic individuals. Present study attempts to establish association of glycosylated hemoglobin (HbA1C) with NAFLD and its severity, compared with body mass index (BMI), waist to hip ratio (WHR) and waist circumference (WC). **Methods:** This case control study was conducted on a sample of 450 individuals including 150 cases and 300 age and gender matched controls recruited from Dow Radiology Institute through convenient sampling. Severity of the disease among cases was determined upon ultrasonic evidence. Detailed history and physical examination was recorded. HbA1C was determined by TINIA method. The study was part of a project funded by Higher education of Pakistan. **Results:** Among the cases 66% whereas 32% of controls had HbA1C levels more than normal value (5.7%). HbA1C, type II DM and BMI were significantly associated with NAFLD (cOR= 4.12, 2.88, 2.25 (overweigh) and 4.32 (obese) respectively)..WC was found significantly associated with NAFLD in both genders (cOR in males= 5.50 and females= 5.79, $p<0.01$). After adjustment with BMI and Type II DM, HbA1C was found significantly associated with NAFLD (aOR=3.40, $p<0.001$) along with BMI more than 30Kg/m² (aOR= 3.30, $p<0.001$). After stratification of data on the basis of gender HbA1C and WC remained significant (aOR= 2.08 and 2.91, $p<0.05$ respectively) while BMI became insignificant (aOR= 1.65 and 1.73, $p>0.05$ for overweight and obese) in males. In females, HbA1C, WC and BMI (only in obese individuals) were found significantly associated (aOR=5.20 $p<0.001$, 4.28 $p=0.035$ and 2.90 $p=0.029$) respectively. AUC for HbA1C was valid for total population (0.76, CI; 0.715 – 0.809) males (0.706, CI; 0.629 – 0.783) and females (0.80, CI; 0.741 – 0.858). **Conclusion:** HbA1C level is significantly associated with presence of NAFLD and possesses a valid predictability value in both diabetic and non-diabetic individuals. It is the single risk factor that is strongly associated with NAFLD after adjustment with BMI, WHR and WC. HbA1C may be presented as a potential biomarker for NAFLD examined with WC in adult population. Evaluation of HbA1C for potential biomarker for NAFLD merits more studies in other settings.

Background

Non Alcoholic Fatty Liver Disease (NAFLD) refers to a condition where excess fat accumulates in the liver of the people with no history of alcohol consumption. Fat molecules are deposited in the form of triacylglycerol (TAG) in hepatocytes. NAFLD is alarmingly increasing round the globe. It is known to progress into Non-Alcoholic Steatohepatitis (NASH), followed by cirrhosis of liver and eventually failure or development of carcinoma of liver[i]. The exact mechanism of the development of both NAFLD and subsequently NASH needs to be further explored. Type II Diabetes Mellitus (Type II DM), insulin resistance, obesity and dyslipidemias are the some of the known risk factors and comorbidities[ii]. In Type II DM, insulin resistance results in high blood glucose levels with an increased tendency to glycosylate the plasma proteins non-enzymatically resulting in formation of glycosylated hemoglobin (HbA_{1C}) and other advanced glycation end products (AGEs). As this reaction is irreversible, HbA_{1C} once formed, persists till the survival of red blood cells (RBC) (Mean 116 and 106 days in males and females respectively).

The estimated global prevalence of NAFLD ranges from 6.3 – 33% among general population[[iii](#)]with an increasing trend worldwide[[iv](#)]. It is the most common chronic liver disease in USA (25-30%) and Asia Pacific region (13-60%). The reported figures from Asian countries are from India 5 – 28%, China 5 – 24%, Japan 9 -14%, Hong Kong 16%, Taiwan 11 – 41% and Indonesia 30%[[v](#)]. In this context only a few studies have been conducted in Pakistan. A tertiary care hospital based study involving 925 healthy population excluding high risk factors like obesity and diabetes found 129 subjects suffering from NAFLD accounting for 13.9%[[vi](#)]. It varies among and within populations. The prevalence is highest among obese (57%) and diabetic (90%) populations[[vii](#)]. According to American Diabetic Association (ADA) an acceptable range of HbA_{1c} in Diabetics is <6.5 % DDC and reflects good blood glucose control[[viii](#)]. In Diabetic patients HbA_{1c} levels are positively correlated with the risk of CVD, retinopathies, nephropathies and neuropathies. Advanced glycation end products (AGEs) have been implicated in the pathogenesis of these complications[[ix](#)].

Generally high levels of HbA_{1c} indicate inadequate blood glucose control. As NAFLD has been reported to be strongly associated with type II DM[[x](#)], it is likely that high levels of HbA_{1c} will be found in these cases. Along with BMI and WHR, central obesity has been linked to insulin resistance and type II DM[[xi](#)],[[xii](#)]. The debate on the relevance of various body weight measurements including BMI, WHR and WC has generated much data with conflicting observation regarding their significance as the risk factor for NAFLD[[xiii](#)],[[xiv](#)],[[xv](#)]

It is therefore tempting to speculate that simultaneous non-enzymatic glycosylation of enzymes involved in glucose and lipid metabolism may trigger lipid accumulation in liver even in the absence of diagnosed diabetes mellitus. Hence raised HbA_{1c} may be a useful biomarker of NAFLD. Moreover the various indices of body weight measurements as known risk factors of NAFLD may also be compared with the association of HbA_{1c} with NAFLD. This case control study was designed (1) to measure association of HbA_{1c} with its severity and (2) Compare the associations of HbA_{1c} with BMI, WHR and WC in the study population.

Methods

This case control study was conducted at Dow University of Health Sciences (DUHS). Individuals undergoing upper abdominal ultrasonography (U/S) at Department of Radiology DUHS were recruited for the study. Those having fatty liver disease on ultrasound were identified as cases, while people showing no fatty infiltration were included as control. 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 Pakistan⁶, sample size was calculated by OpenEpi as 104 (52 each in case and control group). However to improve strength of 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 & gender matched controls). The severity of FLD was graded on the basis of fatty infiltration

found on ultrasonography, from grades (1-3) Grade 1: Minimal infiltration when echogenicity is slightly increased, Grade 2: Moderate infiltration with echogenically obscured portal vessel walls and Grade 3: Heavy fatty infiltration[i].

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, and those having history of alcohol consumption were excluded. Patients with type 1 diabetes mellitus and individuals with known NAFLD were also excluded. History regarding presenting complaints, comorbidities, lifestyle, dietary intake and medication were recorded on structured performa. Detailed physical examination was carried out. Height in meters and weight in Kg was recorded for BMI calculation as Kg/m^2 , waist circumference (WC) and waist-hip ratio (WHR) were measured by standard method¹¹. Blood samples were collected in fasting state in appropriate containers with bar code, for estimation of blood glucose (FBS) by enzymatic method expressed in mg/dl and HbA_{1c} by turbidimetric inhibition immunoassay (TINIA) expressed in percentages (%). The value 5.7% and below was taken as normal[ii]. The study was approved by institutional review board DUHS (IRB-447/DUHS/-14) and funded by Higher Education Commission Pakistan.

Statistical Analysis:

Data was analyzed using the software 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 Chi-square (χ^2) test, while mean differences for anthropometric measures with NAFLD severity grades were assessed using ANOVA. Binary logistic regression (univariate and multivariate) were used to analyse the factors associated with the occurrence of NAFLD. Results of regression were reported as crude and adjusted odds ratio (OR) and 95% confidence interval (CI). ROC curve was plotted to compare each variable with NAFLD. P value < 0.05 was taken as significant.

Results

Baseline characteristics are given in Table-1. Females dominated the sample with 56% in cases and 60.3% in controls (Table-1). 43.96 ± 11.06 years was recorded as the mean age of study sample.

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

Odds for HbA_{1c} was significantly high (cOR= 4.12 (2.72-6.25)) and it was found to be consistently high after adjusting with history of Type II DM and the indices of body measurements (BMI, WHR and WC)

3.40 (2.19-5.26), in males (2.08 (1.06-4.11)) and females 5.20 (2.79-9.68) (Table-3). BMI was significantly associated with NAFLD, however after adjusted with Type II DM and HbA_{1C}, the odds of BMI were found significant only in obese individuals, further after stratification of data on the basis of gender it became insignificant in males. In both genders WHR was found not significant. Odds for HbA_{1C} and WC were found consistently significant in total study sample as well as in both genders (Table-3).

Receiver operating Characteristic (ROC) curve demonstrated valid prediction value of HbA_{1C} in comparison with WC, HbA_{1C} for a binary outcome (NAFLD) (Figure A&B) in both genders. In total study population AUC of HbA_{1C} was 0.7624(95%CI: 0.715 – 0.809). A significant difference was found in males (AUC=0.7066, 95% CI: 0.629 – 0.783) and females (AUC=0.8001, 95%CI: 0.741 – 0.858).

Discussion

Non-Alcoholic Fatty Liver disease (NAFLD) is being recognized as a major global health issue in both alcoholic and non-alcoholic persons, affecting all populations irrespective of age, gender, BMI and ethnicity[i]. It is one of the three most common chronic liver diseases worldwide, the other two being alcoholic liver diseases and viral hepatitis[ii]. Apparently taken as benign disease, NAFLD has now become one of the common indications for liver transplantation in the western world[iii]. Various diagnostic tests have been used for the diagnosis of NAFLD and assessment of severity like US, CT, MR, and fibroscan[iv]. Liver biopsy still remains the gold standard for diagnosing and identifying NAFLD and NASH. Albeit it is seldom used[v] because of invasiveness, correct assessment of time of use and affected persons' will. Attempts are in progress to develop noninvasive methods to predict NAFLD. This has become more important due to the fact that numerous drug trials are underway, some in phase IV. Follow up of these cases is not feasible with multiple biopsies. FLD is a slow progressing disease which rarely exhibits any discomfort. This leads to an un-noticed pathological / biochemical change in liver parenchyma of affected individual that culminates into serious outcomes. The chemical variable selected for this study represented glycemic control (HbA_{1C}) one of the advanced glycation end-products (AGEs).

Advance Glycosylation end-products (AGE) are implicated in development of DM and coronary artery disease[vi]. HbA_{1C} is produced in direct proportions of blood glucose concentrations[vii]. Deranged carbohydrate metabolism, also effects lipid metabolism and results in increased synthesis of triacylglycerol (TAG) that tends to deposit in various tissues of body including liver. TAG deposition in adipose tissue increases BMI while in liver parenchyma it leads to fatty liver. DM has strongly been linked with fatty deposition in liver and HbA_{1C} may be causally associated with NAFLD[viii]. On the other hand obesity in absence of DM also relates to increased fat content of the body tissues. Higher BMI has been associated with insulin resistance and increases in HbA_{1C} [ix]. Patient suffering from NAFLD show a higher level of HbA_{1C} independent of DM[x]. Various body measurements along with different chemical markers have been used in different combinations to develop noninvasive diagnostic tools like fatty liver index (FLI), Bard score, Fib4, Steatosis score, NAFLD fibrosis score, NAFLD liver fat score and APRI score¹⁷ with variable sensitivity and specificity.

Present study reports significantly higher HbA_{1c} levels in individuals with NAFLD ($p < 0.001$, Table-1) that increased with grades of severity (Table-2). Chronic hyperglycemia results in non-enzymatic glycosylation of various proteins that may trigger the immune response with consequent subclinical inflammation of soft tissues including liver[xi]. Glycosylated Hemoglobin reflecting long term glycemic control has been studied for its association with NAFLD. A study has reported conflicting results that “increased pancreatic echogenicity is associated with deteriorating glycemic parameters and higher risk of glycemic progression and incident diabetes, independent of HbA_{1c} concentration and NAFLD”[xii]. Recently it has been claimed that hemoglobin glycation index (difference between observed and predicted HbA_{1c} levels based on plasma glucose levels) can identify the non-diabetic individuals at higher risk of developing fatty liver[xiii]. Present study reports a significant association of HbA_{1c} with NAFLD. This association was positive both in diabetic and non-diabetic, obese and lean persons ($cOR = 4.12$, $p < 0.001$). This indicates that those who have HbA_{1c} higher than 5.7% are 4 time more prone to develop fatty liver disease. Similarly a Chinese study has also reported HbA_{1c} as an independent risk factor for development of NAFLD in elderly people¹⁶. Moreover 11.55% (52 individuals) of the study sample were diagnosed as Type II diabetic at the time of recruitment and in concordance with others⁷ more than 90 % of individuals with Type II DM had NAFLD. While 99 (66%) had more than normal HbA_{1c} (Table-1). This indicates that more than half of the individuals did not know about their high HbA_{1c} which may have been in pre-diabetic¹⁷ group and continued with their lifestyle and ended up in FLD. This finding signifies need of more studies to establish the importance of routine HbA_{1c} examination even in otherwise adult healthy individuals.

Various studies have demonstrated variable results when comparing effect of age, gender, BMI & Obesity. There are opinions that BMI is not a good indicator of chronic disease association as compared to abdominal fatness[xiv] (central obesity represented by WC). Excess abnormal fat predisposes to obesity related disease regardless of total body fat. Present study found both BMI and WC significantly different ($p < 0.001$) with presence of NAFLD in both genders (Table-1) while WHR was significantly different only in 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, 4.28, $p < 0.001$ respectively). This indicates that abdominal obesity is more associated with presence of NAFLD. It has been reported that people with central obesity have less activity index[xv]. Central obesity has been found more prevalent in subcontinent[xvi],[xvii]. Even the patients, who are lean, develop fatty liver if they have central obesity[xviii]. Both of these conditions are associated with insulin resistance and hence high HbA_{1c} may be a common link between NAFLD and DM /center obesity. Insulin resistance predisposes to type 2 DM, Obesity, Central Obesity and increased TAG. The results of this study also depicted that as compared to male patients, females had higher central obesity (Table-1) and NAFLD. Present study demonstrates that NAFLD can be predicted by a combination of HbA_{1c} and WC both in males ($AUC = 0.706$ & 0.681) and in females ($AUC = 0.800$ & 0.632 respectively). It is in concordance to others who claimed that a combination of age, sex, waist circumference, ALT, HbA_{1c}, and HOMA-IR with an AUC of 0.87 can best predict NAFLD[xix].

This study also demonstrated significant increases in various severity grades of fatty liver with the rise in HbA_{1c} levels, BMI and WC (Table-2). Increasing insulin resistance has been reported with higher grades of NAFLD in diabetic and pre-diabetic individuals[xx] while other have claimed that glycated albumin/glycated hemoglobin is inversely proportional to the severity grades of NAFLD[xxi]. With this data it is tempting to speculate that investigation of HbA_{1c} and central obesity may give an insight to the presence of NAFLD well before it is diagnosed.

Conclusions

Present study provides substantial evidence that high HbA_{1c} level is significantly associated with presence of NAFLD in both diabetic and non-diabetic individuals. HbA_{1c} is the single risk factor that is strongly associated with NAFLD and its severity. Among the indices of body measurements WC was found a strong risk factor in both genders, BMI was only significant in obese females while association of WHR with NAFLD was not found significant in both genders. HbA_{1c} may be presented as a potential biomarker for NAFLD examined with WC in adult population. Evaluation of HbA_{1c} for potential biomarker for NAFLD merits more studies in other settings.

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. And therefore some of the potential cases may have been grouped as controls.

Declarations

- **Ethical approval and consent to practice:** The study was approved by institutional review board DUHS (IRB-447/DUHS/-14)
- **Consent for publication:** Not applicable
- **Availability of data and material:** Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.
- **Competing interests:** None of the authors have competing interests.
- **Funding:** The study is supported by Higher education Commission (HEC) of Pakistan by financial grant (No. 20-4231/NRPU/R&d/HEC/14). Role of HEC is to support the research study only.
- **Author's Contributions:** Muhammad Masroor; conception and design of study, recruited and examined patients, collected data, performed the assays, analyzed data and wrote paper; Zeba Haque; conception and design of study analyzed data and wrote the paper.
- **Acknowledgments:** Authors are highly obliged sonologists at Institute of Radiology and Ms Sidra Zaheer at Department of Research Dow University of Health Sciences for their cooperation to sample recruitment and analysis of data respectively.

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Tables

Table 1: Baseline characteristics among cases and controls (n=450)

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

WC $\geq$ 80 cm	81 (96.4)	149 (82.3)
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HbA_{1C}: Glycosylated hemoglobin, BMI: Body Mass Index, WHR: Waist to Hip Ratio, WC: Waist circumference

Table 2: Variations HbA1C and Indices of Body Measurements with severity of NAFLD

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

HbA_{1C}: Glycosylated hemoglobin, BMI: Body Mass Index, WHR: Waist to Hip Ratio, WC: Waist Circumference, NAFLD: Non Alcoholic Fatty Liver Disease

Table 3: Associations HbA_{1C}, BMI, History of Type II DM, WHR and WC with NAFLD

		NAFLD		NAFLD-Male		NAFLD-Female	
		cOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
HbA_{1C}							
	≤ 5.7%	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
Diabetes status							
	No	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
BMI							
	Underweight/Normal	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
	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
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)
Waist circumference							
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)

cOR: crude odds ratio, CI: confidence interval, aOR: adjusted odds ratio for variables HbA_{1C}, Diabetes status, BMI, WHR and WC; however aOR for WHR and WC were calculated after stratification on the basis of gender.

HbA_{1c}: Glycosylated hemoglobin, BMI: Body Mass Index, WHR: Waste to Hip Ratio, WC: Waist Circumference, NAFLD: Non Alcoholic Fatty Liver Disease

Figures

Figure 1

Receiver Operating Characteristic Curve of HbA1c and waist circumference in Males (A) and Females (B) in relation to NAFLD.