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Determination of Insulin Resistance and Beta-Cell Function Using Homeostatic Model Assessment in Type 2 Diabetic Patients at Diagnosis

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Abstract

Objective: Despite the increasing incidence of type 2 diabetes mellitus (T2DM) in South Asian countries, there have been no studies related to insulin resistance (IR) and beta-cell function (BCF) in Nepal. Measurement of both of these parameters at T2DM diagnosis can be a potential tool in evaluation, risk stratification and monitoring treatment. In this study, we used C-peptide modified homeostatic model assessment (both HOMA1 and HOMA2) and correlated the obtained IR with cardiovascular risk factors. We also intended to find out whether reduced insulin sensitivity or beta-cell failure predominates in new T2DM cases. Also there has been some dilemma in using either body mass index (BMI) or waist-hip ratio (WHR) as a better predictor of IR in our population. Lipoprotein ratios TC/HDL and TG/HDL also needed evaluation in this regard.

Results: Participants were sixty newly diagnosed T2DM patients visiting Tribhuvan University Teaching Hospital (TUTH), Nepal. The mean IR and beta-cell function were HOMA1IR=4.91 ± 1.62; HOMA2IR=2.61 ± 1.06 and HOMA1%B=40.28 ± 23.64; HOMA2%B=47.10 ± 24.67 respectively. Both HOMA1 and HOMA2 showed greater reduction in insulin sensitivity than beta-cell function at diagnosis. ROC curves analysis showed WHR and TC/HDL ratio as better predictors of IR.

Keywords: Beta cell function; C-peptide; Cardiovascular risk factors; Diabetes mellitus; Insulin resistance; HOMA

Abbreviations:

%B: Beta-Cell Function; %S: Insulin Sensitivity; AUC: Area Under the Receiver Operating Characteristic Curve; BCF: Beta-Cell Function; BMI: Body Mass Index; DECODA: Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Asia; DECODE: Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe; FPG: Fasting Plasma Glucose; FBG: Fasting Blood Glucose; FPI: Fasting Plasma Insulin; HDL-C: High-Density Lipoprotein-Cholesterol; HOMA: Homeostasis Model Assessment; IR: Insulin Resistance; ISG: Insulin Sensitivity Group; LDL-C: Low-Density Lipoprotein Cholesterol; ROC: Receiver Operating Characteristic; T2DM: Type 2 Diabetes Mellitus; TUTH: Tribhuvan University Teaching Hospital; WHR: Waist–Hip Ratio

Introduction

Insulin resistance (IR) and beta-cell dysfunction are characteristic features of type 2 diabetes mellitus (T2DM). Insulin resistance is characterized by decrease in insulin mediated glucose disposal in insulin-sensitive tissue and increased hepatic glucose production [1] whereas beta-cell dysfunction occurs when beta-cells are unable to compensate for the insulin resistance [2]. Measurement of both of these parameters at diagnosis of T2DM can be a potential tool in evaluation, risk stratification and monitoring treatment of DM.

Hyperinsulinemic-euglycemic clamp technique is the gold standard method for determining IR. However it is cumbersome to perform, labor-intensive and costly [3]. Homeostatic Model Assessment (HOMA) test introduced in 1985 by David Matthews et al. is a simple test that mathematically models the fasting plasma glucose and insulin concentrations and gives an estimate of individual's degree of insulin sensitivity (HOMA %S) and level of beta-cell function (HOMA %B). HOMA-IR is the reciprocal of HOMA %S [4]. In 1998, Jonathan Levy et al published an updated HOMA model (HOMA2) which along with the steady state glucose and insulin ratio also considers variations in hepatic and peripheral glucose resistance i.e., the reduction in the suppression of hepatic glucose output (by hyperglycemia), increases in the insulin secretion curve for plasma glucose concentrations above 10 mmol/L and the effects of circulating proinsulin [5]. Later, in 2004, a computer model called the HOMA Calculator was released which could provide quick and easy access to the HOMA2 model [6].

However, these original models which use insulin have some limitations. First, the insulin secretion is pulsatile, which limits the use of a single sample for insulin determination. Instead, we had to use the mean of at least three samples taken at 5-min intervals to compute HOMA for more reliable results. Also careful phlebotomy is essential to prevent hemolysis as far as possible as hemolysis results in degradation of insulin. Moreover, nearly 50% of insulin secreted by beta-cells are extracted by the liver causing a large insulin inter assay variation [7].

In China, Li et al. [8] found a modified homeostasis model assessment (modified HOMA) using C-peptide to replace insulin in HOMA to evaluate both IR and islet cell function in normal and diabetic patients. This C-peptide modified HOMA seems more appropriate as a measure of insulin secretion as the equimolarly secreted C-peptide is not extracted by the liver and other organs and also, the half-life of C-peptide in blood is quite longer than that of insulin (10-30 min vs. 4 min) making

the measurement of C-peptide a more reliable representative of beta-cell insulin secretion compared to the measurement of insulin itself [2].

Insulin resistance has been well known as the major player in progression from metabolic syndrome to overt diabetes mellitus. IR as assessed by HOMA indices has also been correlated with intima-media thickness of the carotid artery [9] which is a well-accepted marker of atherosclerosis. Also, the lipid markers like plasma triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) have been reported to be independently associated with insulin resistance, and are independent predictors of cardiovascular disease (CVD) [10]. Lipoprotein ratios such as TC/HDL-C and TG/HDL-C ratios which are derived from these lipid markers, have been shown by several epidemiological studies as better predictors of CVD than any single lipid marker [11-13]. One of the major advantages of these ratios is that they can be promptly calculated by the physician and are easier to comprehend at the clinical level.

Studies have also shown that, it takes 5-10 years before actual symptoms of diabetes start to appear due to defect in beta-cell function (BCF) and insulin resistance. Under such scenario, early detection of insulin resistance and beta-cell function could be of potential benefit for applying preventive measures to attenuate the progression to overt diabetes. Various dynamic and steady state tests are available for this but each has their own pros and cons. There is a paucity of nationwide data regarding this. So this study has been designed with the following objectives:

•To determine insulin resistance and beta-cell function using modified HOMA in newly diagnosed type 2 diabetic patients.

•To know whether reduced insulin sensitivity or beta-cell failure predominates in newly diagnosed type 2 diabetic patients.

•To correlate insulin resistance with BMI and waist hip ratio (WHR).

•To correlate insulin resistance and beta-cell function with other cardiovascular risk factors such as hypertension, family history of DM, dietary habit, stress, exercise, alcohol intake, smoking, tobacco intake, and lipid profile.

We also investigated the reliability of TC/HDL-C and TG/HDL-C ratios as surrogate marker of insulin resistance.

Materials and Methods

Place and duration of study

This study was conducted at Department of Endocrinology and Biochemistry Laboratory of Tribhuvan University Teaching Hospital (TUTH), a tertiary care centre in Kathmandu, Nepal from February 2016 to January 2017.

Study population, inclusion and exclusion criteria

Sixty newly diagnosed T2DM patients who provided written consent were enrolled in this study. Exclusion criteria included patients with evident major diabetic complications, such as heart disease and diabetic nephropathy, patients suffering from chronic illness, chronic liver and/or renal diseases, patients already receiving lipid-lowering medications and/or oral hypoglycemic drugs, and pregnant women. Participants were interviewed by the principal investigator by asking the questions included in questionnaire (self-designed semi-structured proforma). Thirty age and sex matched non-diabetic apparently healthy individuals were recruited as control.

Anthropometric measurements

Weight was taken using a platform weighing scale. Standing height measurement was done with participants in bare foot, eyes looking ahead. The waist circumference was measured at the midpoint between the lowest rib and iliac crest and at the end of expiration. The hip circumference was taken at the widest area of the hips at the greatest protuberance of the buttocks. BMI was calculated by weight in kilograms divided by square of height in meters. WHR was calculated by simply dividing the waist measurement by the hip measurement. Blood pressure (BP) measurement was done using a recently calibrated aneroid sphygmomanometer with an adequate cuff size after participant has rested for at least 5 minutes.

Collection and processing of the sample

Five millilitre of blood was drawn after an overnight fast (8 -12 hours) by venous puncture method. Serum samples were separated, within half an hour, by centrifugation at 1500 - 3000 rpm for 5 min. Routine investigation were done on the same day of sample collection, which included blood glucose, creatinine, SGPT, TC, HDL-cholesterol and TG, which were measured in fully-automated biochemistry analyser, BT 3000, Italy. An aliquot of each sample was then stored at -20°C for the test of C-peptide.

Laboratory standard operating procedures were maintained for all laboratory analysis. Internal quality control sera, both normal and pathological, were also run for each lot, for the validation of the results.

Fasting serum glucose was measured by glucose oxidase method, as described by Trinder, using commercial kit Biolabo Reagents, France. Serum creatinine was measured by modified Jaffe reaction, Biolabo Reagents, France. SGPT was measured by IFCC recommended kinetic method. Total cholesterol (TC) was estimated by CHOD/PAP method, Human, Germany. Triglyceride (TG) was measured by GPO/PAP method, Human, Germany. HDL-C was measured by PEG/CHOD-PAP method, Human, Germany. LDL-C was calculated using the Friedewald's formula:- LDL-C (mmol/L) = TC (mmol/L) – HDL-C (mmol/L) – TG (mmol/L)/2.2. When TG concentration exceeded 4 mmol/l, LDL-C was estimated by direct homogenous method, Biolabo Reagents, France. VLDL-C (mmol/L) was calculated as TG (mmol/L)/2.2.

C-peptide was measured using a solid phase enzyme-linked immunosorbent assay (ELISA) kit, DRG, Italy. The HOMA model was used to calculate insulin resistance, insulin sensitivity, and beta-cell function using the C-peptide modified formulae given by Li et al. [8]

The modified HOMA formulas were:-

HOMA1-IR = 1.5 + FPG × FCP / 2800

HOMA1 %B (Normal) = 0.27 × FCP / (FPG-3.5) + 50

HOMA1 %B (DM) = 0.27 × FCP / (FPG - 3.5)

where,

FPG = Fasting plasma glucose (mmol/L)

FCP = Fasting C-peptide (pmol/L)

HOMA1 %S was calculated by reciprocal of HOMA1-IR multiplied by 100%. Patients with HOMA1-IR > 2.5 were defined as having insulin resistance whereas those with HOMA1-IR ≤ 2.5 were defined as insulin sensitivity group [13,14]. IR and BCF values were also calculated using the HOMA2 calculator software. HOMA2 calculator was downloaded from university of oxford. http://www.dtu.ox.ac.uk/. Patients with

HOMA2-IR > 1.8 were defined as having insulin resistance whereas those with HOMA2-IR ≤ 1.8 were defined as insulin sensitivity group (ISG) [15]. The calculated HOMA1-IR and HOMA2-IR were compared in individual patients. HOMA2 being more accurate representation of glucose homeostasis than HOMA1 [7], was used to compare various physical, socio-demographic, laboratory and clinical parameters between the insulin resistant and ISG of the diabetic cases in this study. Diabetic patients were also separated into two groups on the basis of presentation with osmotic symptoms (polyuria, polydipsia, polyphagia, and dehydration) and without osmotic symptoms and these two groups were compared for biochemical parameters.

Data processing and analysis

The data were entered in Microsoft Excel program (Microsoft Office 2010). Statistical analyses were done by SPSS 23.0 version (Statistical Package for Social Science for Window version; SPSS, Inc., Chicago, IL). Mean comparison was done by t-test. Chi square test was used for comparison of dichotomous variables. Pearson correlation was used to evaluate the correlation between cardiovascular risk factors and insulin resistance. P value ≤0.05 was considered to be statistically significant.

Evaluation of serum lipoprotein ratios was done by constructing receiver operating characteristic (ROC) curve to examine the predictive value of TC/HDL-C, and TG/ HDL-C ratios for insulin resistance (HOMA2-IR > 1.8). Values for the area under the ROC curve of 0.5, ≥ 0.7 but <0.8, ≥ 0.8 but < 0.9, and ≥ 0.9 were taken as suggestive of reflecting the following levels of discrimination: none, acceptable, excellent, and outstanding [16].

Results

The mean age of type 2 diabetic patients at diagnosis was 46.0 ± 7.8 years (n=60). The age ranged from 33 years to 66 years. This study population included 36 males and 24 females. The mean age for male patients was 44.9 ± 8.4 years and for female patients was 47.7 ± 6.6 years. The mean age of control individuals was 44.5 ± 8.4 years (n=30). Basic characteristics of case and control are depicted in Table 1. Fasting blood glucose, fasting C-peptide, insulin resistance as assessed by HOMA1 and HOMA2, serum TG, TC/HDL-C ratio, and TG/HDL-C ratio were significantly higher in cases than controls; whereas insulin sensitivity and beta-cell function as assessed by HOMA1 and HOMA2 were significantly lower in cases than controls. Both HOMA1 and HOMA2

showed greater reduction in insulin sensitivity (i.e. higher insulin resistance) than reduction in beta-cell function in diabetic cases (Table 1). This indicates that the development of insulin resistance proceeds over deterioration of beta-cell secretory capacity in newly diagnosed type 2 diabetic patients in our settings.

Among 60 diabetic cases, HOMA1 identified 56 patients as insulin resistant and only 4 patients as insulin sensitivity group with the cut off of 2.5 whereas HOMA2 identified 43patients as insulin resistant and 17 patients as insulin sensitivity group with a cut off of 1.8. Concordant results were obtained for 47 patients whereas 13 HOMA1 insulin resistant patients were deemed under insulin sensitivity group by HOMA2 (Table 2). HOMA2-IR gave comparatively lower indexes than HOMA1-IR. Scatter plot relationship between HOMA1 and HOMA2 is shown in Figure 1. Using HOMA2, significant mean differences in insulin resistant and insulin sensitive diabetic groups were found for LDL cholesterol and waist hip ratio (Table 3) and for age group, presence of osmotic symptoms, family history of diabetes, adequacy of vegetables intake, and stressful lifestyle (Table 4). Similarly, between patients with osmotic symptoms and without osmotic symptoms, significant difference in means were obtained for FBG, FCP, HOMA1-IR, HOMA1 %S, HOMA1 %B, HOMA2-IR, HOMA2 %S and HOMA2 %B (Table 5). Higher insulin resistance was seen in patients presented with osmotic symptoms than those without osmotic symptoms (Figure 2). Significant positive correlations were obtained in both HOMA1-IR and HOMA2-IR for LDL cholesterol, waist hip ratio, blood pressure, family history of diabetes and stress (Table 6). Oil intake correlated significantly with HOMA1-IR and tobacco intake correlated significantly with HOMA2-IR. Similarly, significant negative correlations were obtained in both HOMA1 %B and HOMA2 %B for LDL cholesterol, family history of diabetes, oil intake, salt intake and stressful lifestyle (Table 7). Exercise had significant positive correlation with both HOMA1 %B and HOMA2 %B and alcohol intake had significant negative correlation with HOMA2 %B.

Finally, ROC curves were drawn for both BMI and WHR for prediction of insulin resistance (Figure 3). HOMA2-IR was used as gold standard (>1.8 was taken as positive for insulin resistance). The AUC for prediction of insulin resistance for WHR was significantly higher than for BMI (Table 7). Lipoprotein ratios: TC/HDL-C and TG/HDL-C were also analyzed using ROC curves for prediction of insulin resistance (Figure 4a and 4b). TC/HDL-C ratio was found to have acceptable

	Case Diabetic patients) n=60		Control(Non-diabetic; apparently healthy individuals) n=30		p-value
	Mean	SD	Mean	SD	
Age (years)	46.0	7.8	44.5	8.4	0.395
Fasting Blood Glucose (mmol/l)	10.68	3.46	4.90	0.52	<0.001*
Fasting C-peptide (pmol/l)	875.41	294.53	534.25	143.22	<0.001*
HOMA1-IR	4.91	1.62	2.43	0.26	<0.001*
HOMA1 %S	22.84	8.25	41.57	4.76	<0.001*
HOMA1 %B	40.28	23.64	168.60	53.69	<0.001*
HOMA2-IR	2.61	1.06	1.16	0.31	<0.001*
HOMA2 %S	46.34	22.81	93.91	32.61	<0.001*
HOMA2 %B	47.10	24.67	113.10	30.56	<0.001*
Total Cholesterol (mmol/l)	4.55	0.96	4.21	0.68	0.086
Triglyceride (mmol/l)	2.33	1.43	1.18	0.56	<0.001*
HDL Cholesterol (mmol/l)	1.00	0.22	1.06	0.16	0.249
LDL Cholesterol (mmol/l)	2.68	0.87	2.59	0.50	0.526
TC/HDL-C ratio	4.60	0.93	4.00	0.49	<0.001*
TG/HDL-C ratio	2.37	1.61	1.15	0.59	<0.001*

*Statistically significant at p<0.05; Independent Sample t test

Table 1: Comparison of different physical and laboratory parameters between case (diabetic patients) and control (apparently healthy individuals).

Patient S. No.	HOMA-IR	Categorization based on cut-off of 2.5	HOMA2-IR	Categorization based on cut-off of 1.8	Concordance between HOMA-IR and HOMA2-IR
1	6.22	IR	4.59	IR	Yes
2	5.48	IR	2.80	IR	Yes
3	5.94	IR	3.18	IR	Yes
4	4.66	IR	2.77	IR	Yes
5	5.89	IR	3.00	IR	Yes
6	7.68	IR	4.03	IR	Yes
7	6.34	IR	3.40	IR	Yes
8	5.49	IR	3.82	IR	Yes
9	4.36	IR	2.69	IR	Yes
10	3.82	IR	2.22	IR	Yes
11	3.75	IR	1.74	ISG	No
12	3.23	IR	1.50	ISG	No
13	2.95	IR	1.29	ISG	No
14	5.59	IR	2.77	IR	Yes
15	5.13	IR	2.88	IR	Yes
16	3.42	IR	1.69	ISG	No
17	6.02	IR	3.00	IR	Yes
18	3.93	IR	1.73	ISG	No
19	3.40	IR	1.63	ISG	No
20	4.74	IR	2.20	IR	Yes
21	9.09	IR	5.68	IR	Yes
22	4.31	IR	2.03	IR	Yes
23	2.49	ISG	1.00	ISG	Yes
24	3.20	IR	1.53	ISG	No
25	2.59	IR	1.03	ISG	No
26	6.70	IR	3.75	IR	Yes
27	2.44	ISG	.92	ISG	Yes
28	3.49	IR	2.21	IR	Yes
29	9.39	IR	5.29	IR	Yes
30	5.60	IR	2.72	IR	Yes
31	2.30	ISG	0.93	ISG	Yes
32	4.15	IR	1.93	IR	Yes
33	6.47	IR	3.91	IR	Yes
34	4.33	IR	2.27	IR	Yes
35	6.36	IR	3.36	IR	Yes
36	3.26	IR	1.46	ISG	No
37	4.98	IR	2.48	IR	Yes
38	6.48	IR	3.30	IR	Yes
39	5.01	IR	2.73	IR	Yes
40	3.75	IR	2.39	IR	Yes
41	6.10	IR	3.70	IR	Yes
42	5.07	IR	2.83	IR	Yes
43	5.14	IR	3.22	IR	Yes
44	3.28	IR	1.52	ISG	No
45	6.68	IR	3.44	IR	Yes
46	5.36	IR	2.53	IR	Yes
47	7.64	IR	4.02	IR	Yes
48	4.76	IR	2.76	IR	Yes
49	5.58	IR	3.76	IR	Yes
50	4.84	IR	2.84	IR	Yes
51	2.29	ISG	1.02	ISG	Yes
52	3.73	IR	1.59	ISG	No
53	4.70	IR	2.13	IR	Yes
54	2.84	IR	1.29	ISG	No
55	5.31	IR	2.65	IR	Yes
56	2.77	IR	1.22	ISG	No
57	4.82	IR	2.51	IR	Yes
58	5.58	IR	2.76	IR	Yes
59	6.68	IR	3.55	IR	Yes

60	6.92	IR	3.57	IR	Yes
Total		IR = 56 (93.3%)		IR = 43 (71.7%)	Yes = 47 (78.3%)
		ISG = 4 (6.7%)		ISG = 17 (28.3%)	No = 13 (21.7%)

IR: Insulin Resistant
ISG: Insulin Sensitivity Group

Table 2: Comparison of HOMA1-IR and HOMA2-IR in categorization of individual patients with respective cut-off values and concordance between them.
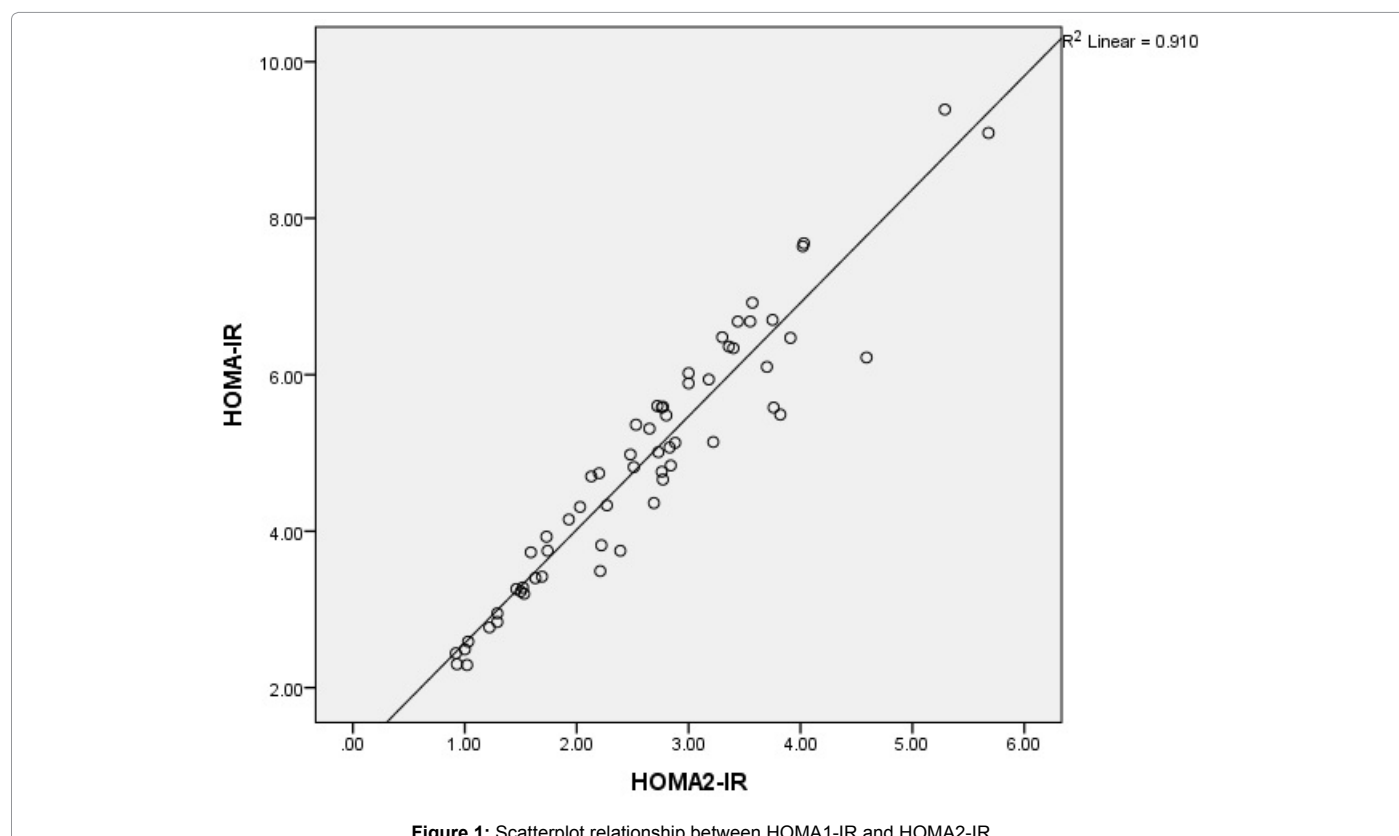

Figure 1: Scatterplot relationship between HOMA1-IR and HOMA2-IR.

	HOMA2-IR ≤ 1.8 (n=17)		HOMA2-IR >1.8 (n=43)		
	Mean	± SD	Mean	± SD	p-value
Age (years)	44.9	9.0	46.5	7.3	0.501
Fasting Plasma Glucose (mmol/l)	8.04	1.75	11.73	3.42	<0.001*
Fasting C-peptide (pmol/l)	529.2	87.6	1012.3	226.3	<0.001*
HOMA-IR	3.05	0.52	5.6430	1.27	<0.001*
HOMA %S	33.71	5.91	18.55	3.95	<0.001*
HOMA %B	36.89	19.82	41.62	25.08	0.490
HOMA2-IR	1.36	0.29	3.11	0.82	<0.001*
HOMA2 %S	77.27	18.22	34.12	8.00	<0.001*
HOMA2 %B	47.91	19.71	46.79	26.58	0.876
Total Cholesterol (mmol/l)	4.33	0.99	4.64	0.95	0.267
Triglyceride (mmol/l)	2.26	1.05	2.36	1.56	0.802
HDL Cholesterol (mmol/l)	1.02	0.23	1.00	0.21	0.777
LDL Cholesterol (mmol/l)	2.33	0.81	2.82	0.85	0.046*
TC/HDL-C ratio	4.32	0.84	4.71	0.95	0.149
TG/HDL-C ratio	2.19	0.83	2.44	1.83	0.484
Body Mass Index (BMI)	27.49	3.75	28.75	3.39	0.214
Waist Circumference (cm)	104.18	10.68	102.65	11.28	0.634
Waist Hip Ratio (WHR)	1.00	0.04	1.04	0.05	0.014*

*Statistically significant at $p<0.05$; Independent Sample t test

Table 3: Comparing different physical and laboratory parameters between patients with HOMA2-IR ≤ 1.8 and >1.8.

		HOMA2-IR ≤ 1.8 (n=17)		HOMA2-IR >1.8 (n=43)		Total	p-value
		No.	%	No.	%		
Age Group	31-40	7	36.8	12	63.2	19	0.012*
	41-50	8	30.8	18	69.2	26	
	51-60	0	0	13	100	13	
	>60	2	100	0	0	2	
Gender	Male	12	33.3	24	66.7	36	0.293
	Female	5	20.8	19	79.2	24	
Obesity[a] (BMI)	Non-obese	6	46.2	7	53.8	13	0.107
	Obese	11	23.4	36	76.6	47	
Obesity[b] (Waist circumference)	Non-obese	2	33.3	4	66.7	6	0.774
	Obese	15	27.8	39	72.2	54	
Obesity (Waist Hip Ratio)	0.80-0.89	0	0	0	0	0	0.079
	0.90-0.99	8	50	8	50	16	
	1.00-1.09	8	20	32	80	40	
	1.10-1.19	1	25	3	75	4	
Osmotic symptoms	No	15	71.4	6	28.6	21	<0.001*
	Yes	2	5.1	37	94.9	39	
Hypertension[c] (BP systolic)	No	17	32.1	36	67.9	53	0.077
	Yes	0	0	7	100	7	
Hypertension[d] (BP diastolic)	No	17	32.7	35	67.3	52	0.056
	Yes	0	0	8	100	8	
Family History of DM	No	15	38.5	24	61.5	39	0.018*
	Yes	2	9.5	19	90.5	21	
Dietary Habbit	Vegetarian	2	66.7	1	33.3	3	0.131
	Non-vegetarian	15	26.3	42	73.7	57	
	2 times daily	4	30.8	9	69.2	13	0.807
	3 times daily	10	30.3	23	69.7	33	
	4 times daily	3	21.4	11	78.6	14	
Exercise	No	16	29.6	38	70.4	54	0.504
	Yes	1	16.7	5	83.3	6	
Vegetables intake[e]	Low	2	9.5	19	90.5	21	0.041*
	Normal	15	39.5	23	60.5	38	
	High	0	0	1	100	1	
Carbohydrates Intake[e]	Low	0	0	1	100	1	0.816
	Normal	5	29.4	12	70.6	17	
	High	12	28.6	30	71.4	42	
Fruits intake[e]	Low	11	32.4	23	67.6	34	0.639
	Normal	6	24	19	76	25	
	High	0	0	1	100	1	
Oil intake[e]	Low	0	0	3	100	3	0.343
	Normal	12	34.3	23	65.7	35	
	High	5	22.7	17	77.3	22	
Salt intake[e]	Low	0	0	4	100	4	0.378
	Normal	15	29.4	36	70.6	51	
	High	2	40	3	60	5	
Lifestyle	Non-Stressful	17	43.6	22	56.4	39	<0.001*
	Stressful	0	0	21	100	21	
Alcohol intake	No	7	28	18	72	25	0.961
	Yes	10	28.6	25	71.4	35	
Smoking	No	14	34.1	27	65.9	41	0.142
	Yes	3	15.8	16	84.2	19	
Tobacco	No	14	30.4	32	69.6	46	0.513
	Yes	3	21.4	11	78.6	14	

*Statistically significant at p<0.05; Chi-square test
[a]Classification was based on two cut-off points of <25 for non-obese and ≥ 25 for obese
[b]Classification was based on two cut-off points for male (≥ 90 cm) and female (≥ 80 cm)
[c]Classification was based on cut-off point of 140 mmHg
[d]Classification was based on cut-off point of 90 mmHg
[e]Classification was based on asking the participant whether he/she prefers taking it in that amount compared to other family members, relatives, or friends.
Table 4: Relation between Insulin Resistance (HOMA2-IR) and Socio-demographic and clinical characteristics.

	Diabetic patients with osmotic symptoms(n=39)		Diabetic patients without osmotic symptoms (n=21)		p-value
	Mean	SD	Mean	SD	
Age (years)	46.8	7.9	44.6	7.5	0.307
Fasting Blood Glucose (mmol/l)	12.29	3.11	7.69	1.59	<0.001*
Fasting C-peptide (pmol/l)	1000.78	248.46	642.58	225.93	<0.001*
HOMA1-IR	5.79	1.23	3.27	0.69	<0.001*
HOMA1 %S	17.98	3.57	31.89	6.67	<0.001*
HOMA1 %B	35.90	19.84	48.41	28.17	0.081*
HOMA2-IR	3.15	0.86	1.62	0.57	<0.001*
HOMA2 %S	34.04	9.25	69.19	22.99	<0.001*
HOMA2 %B	40.58	20.66	59.22	27.33	0.004*

*Statistically significant at $p<0.05$; Independent Sample t test

Table 5: Comparison of different physical and laboratory parameters in diabetic patients with osmotic symptoms and without osmotic symptoms.
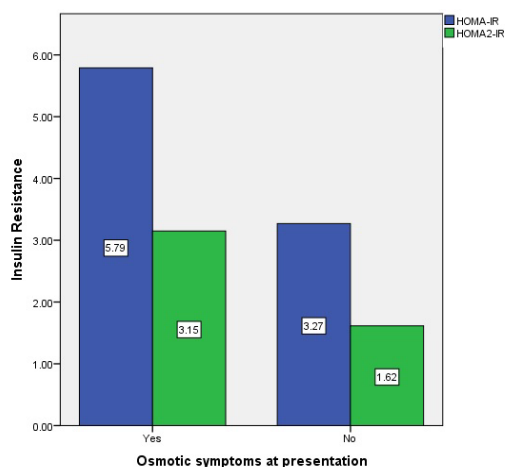

Figure 2: Bar diagram showing higher insulin resistance in cohort of patients with osmotic symptoms at presentation
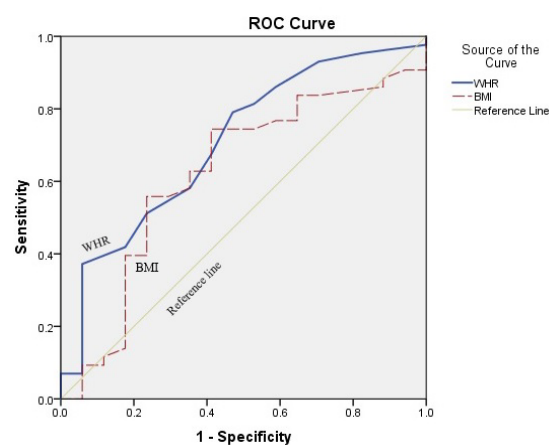

Figure 3: ROC (receiver operating characteristics) curve of BMI and WHR for prediction of patients with insulin resistance.

	HOMA-IR		HOMA2-IR	
	Pearson's Correlation Coefficient	p-value	Pearson's Correlation Coefficient	p-value
Age Group (years)	0.015	0.908	0.002	0.988
Total Cholesterol (mmol/l)	0.266	0.040*	0.237	0.069
Triglyceride (mmol/l)	0.191	0.144	0.177	0.175
HDL Cholesterol (mmol/l)	0.088	0.505	0.084	0.522
LDL Cholesterol (mmol/l)	0.304	0.018*	0.264	0.041*
TC/HDL-C	0.233	0.073	0.203	0.120
TG/HDL-C	0.140	0.287	0.144	0.272
Body Mass Index (BMI)	0.161	0.221	0.141	0.283
Waist Hip Ratio (WHR)	0.284	0.028*	0.274	0.034*
BP systolic (mm Hg)	0.496	<0.001*	0.406	<0.001*
BP diastolic (mm Hg)	0.609	<0.001*	0.562	<0.001*
Family History	0.449	<0.001*	0.335	0.009*
Dietary Habbit	0.128	0.331	0.125	0.340
Dietary Frequency	0.174	0.183	0.183	0.163
Exercise	0.060	0.647	0.089	0.500
Vegetables intake	-0.104	0.428	-0.159	0.226
Fruits intake	0.071	0.591	0.038	0.772
Carbohydrates intake	0.039	0.768	0.018	0.891
Oil intake	0.255	0.049*	0.232	0.075
Salt intake	0.050	0.707	0.005	0.971
Lifestyle	0.683	<0.001*	0.661	<0.001*
Alcohol intake	0.115	0.384	0.068	0.608
Smoking	0.252	0.052	0.205	0.116
Tobacco	0.249	0.055	0.257	0.048*

*Statistically significant at $p<0.05$

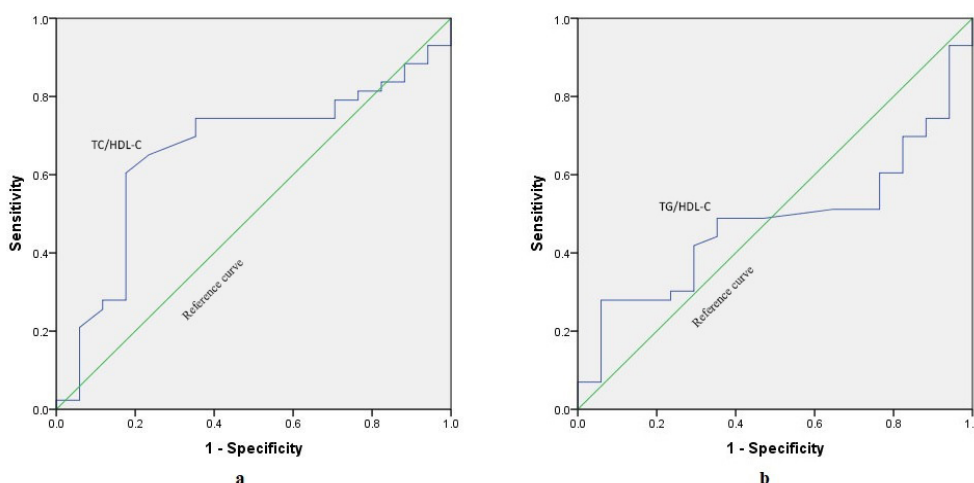**Table 6:** Correlation of insulin resistance with various cardiovascular risk factors.

Figure 4: ROC (receiver operating characteristics) curve of a) TC/HDL-C; and b) TG/HDL-C ratio for detection of patients with insulin resistance.

	HOMA %B		HOMA2 %B	
	Pearson's Correlation Coefficient	*p*-value	**Pearson's Correlation Coefficient**	*p*-value
Age Group (years)	-0.196	0.133	-0.208	0.110
Total Cholesterol (mmol/l)	-0.193	0.139	-0.233	0.074
Triglyceride (mmol/l)	0.085	0.517	0.063	0.630
HDL Cholesterol (mmol/l)	0.000	0.999	-0.008	0.954
LDL Cholesterol (mmol/l)	-0.259	0.046*	-0.301	0.019*
TC/HDL-C	-0.185	0.156	-0.223	0.087
TG/HDL-C	0.134	0.309	0.117	0.372
Body Mass Index (BMI)	-0.030	0.818	-0.058	0.659
Waist Hip Ratio (WHR)	-0.067	0.609	-0.115	0.382
BP systolic (mm Hg)	-0.058	0.659	-0.117	0.372
BP diastolic (mm Hg)	-0.005	0.972	-0.066	0.615
Family History	-0.297	0.021*	-0.342	0.007*
Dietary Habbit	-0.205	0.117	-0.192	0.142
Dietary Frequency	0.144	0.271	0.140	0.288
Exercise	0.351	0.006*	0.309	0.016*
Vegetables intake	-0.224	0.086	-0.193	0.139
Fruits intake	0.079	0.547	0.071	0.591
Carbohydrates intake	-0.019	0.888	-0.039	0.766
Oil intake	-0.348	0.006*	-0.366	0.004*
Salt intake	-0.332	0.010*	-0.307	0.017*
Lifestyle	-0.231	0.076	-0.305	0.018*
Alcohol intake	-0.250	0.054	-0.263	0.042*
Smoking	-0.111	0.397	-0.134	0.309
Tobacco	-0.066	0.616	-0.090	0.496

*Statistically significant at p<0.05

Table 7: Correlation of beta-cell function indices with anthropometric/metabolic variables.

Cardiovascular Risk Factors	Areas under the ROC curve	95% Confidence interval	*p*-value	Optimal cut off for predicting IR
BMI	0.622	0.459 – 0.784	0.144 (NS)	27.70
WHR	0.705	0.559 – 0.851	0.014*	1.015

*Statistically significant at p ≤ 0.05;
NS: Non-significant

Table 8: Comparison of BMI and WHR using areas under ROC (receiver operating characteristic) curve for prediction of insulin resistance (HOMA2-IR).

Serum lipoprotein ratios	Area under the ROC curve	95% Confidence interval	*p*-value	Optimal cutoff for predicting IR
TC/HDL-C ratio	0.70	0.509 – 0.812	0.05*	4.47
TG/HDL-C ratio	0.50	0.329 – 0.625	0.78 (NS)	Indefinable

*Statistically significant at p ≤ 0.05;
NS: Non-significant

Table 9: Serum lipoprotein ratios and the areas under ROC (receiver operating characteristic) curve for detection of insulin resistance (HOMA2-IR).

screening ability for insulin resistance (AUC=0.7; P=0.05) with an optimal cutoff of 4.47 whereas TG/HDL-C ratio failed as a screening tool for insulin resistance as the AUC obtained for it was unacceptable rendering its optimal cutoff indefinable (Table 8).

Discussion

This study demonstrated the magnitude of IR and BCF and their associated metabolic risk factors among the 60 new T2DM attendees of an out-patient department of a tertiary care hospital in Nepal. The mean fasting serum glucose of these recently diagnosed diabetic patients was high (10.68 ± 3.46 mmol/l) and the mean fasting C-peptide was found to be on the upper normal range (875.41 ± 294.53 pmol/l). It has been stated that in early diabetes, glucose regulation is of secondary importance to maintenance of basal insulin secretion. So the basal plasma glucose rises in an attempt to stimulate the reduced numbers of beta cells to secrete normal basal insulin. This forces the remaining beta cells to operate nearer their maximal capacity [17].

Both HOMA1-IR and HOMA2-IR in this study showed significant correlations with WHR, while the correlation between BMI and HOMA1-IR or HOMA2-IR was poor. Mishra et al. from India [18] also found strong correlation of HOMA1-IR with WHR. A similar result was obtained in a large study from Europe [19]. In the Atherosclerosis Risk in Community study [20] also, the AUC of WHR for prediction of IR and T2DM was significantly greater than that of BMI. All these studies have shown that WHR is a good predictor of IR and can thus be used to identify at risk individuals. Although BMI may be a simple non-invasive method for assessing obesity and excessive fat stores, it is less reliable in comparing body composition [21]. One of the reasons for reduced reliability of BMI is that there may be identical distributions of BMI in two populations but they could still reflect large differences with respect to the accumulation of intra-abdominal fat responsible for development of insulin resistance. The findings in this study indicated BMI to be an unreliable measure for associating insulin resistance and T2DM (Table 3). Instead, WHR is a more reliable marker of insulin resistance.

This study also demonstrated the more reduced insulin sensitivity (HOMA %S) compared to reduction in beta cell function (HOMA% B) in new T2DM patients (Table 9). This can be explained by the fact that South Asians have preferential fat deposition in the abdominal region which is associated with reduced insulin-mediated glucose disposal [22]. Thus South Asian population being more prone to abdominal obesity and also low muscle mass, have been hypothesized to develop insulin resistance and consequently type 2 diabetes sooner than the population of European descent [21]. In addition, higher prevalence of low birth weight (indicator of intrauterine malnutrition) along with low muscle mass in their lifetime has also been attributed for the higher prevalence of diabetes in these populations [23]. Poor nutrition in foetal life and infancy predisposes to fewer beta cells and stimulates the development of thrifty genes that confers a survival advantage in such nutrient deficient environments. But it becomes disadvantageous in later life when nutrition becomes abundant and increased demand for insulin outstrips the capacity of production, predisposing the individual to development of T2DM [24,25].

A positive family history for DM is a controversial contributing risk factor for developing IR and DM. Some researchers have demonstrated a significant association between a positive family history of DM and IR [26], whereas other studies strongly disagree [27]. Subjects having positive family history of T2DM are found to have low insulin like growth factor binding protein-1 levels which is associated with adolescent obesity and impaired beta-cell compensation relative to lower insulin sensitivity [26]. Regarding stressful lifestyle as one of the causative factors for development of insulin resistance and subsequent cardiovascular risks, a study done by Räikkönen et al. [28] shows a close correlation of stress related factors and insulin resistance whereas in a study done by Zareian et al. [29], there was no significant correlation between insulin resistance and stress score. This study didn't use any type of questionnaire (eg. Osipow questionnaire as used by Zareian et al.) to assess the level of stress. The role of stress in development of insulin resistance has been associated with accumulation of intra-abdominal fat, hyperlipidaemia, hypertension, altered insulin/glucose homeostasis, and an increased level of plasminogen activator inhibitor-1 [28].

Concerning daily oil intake, the type of fatty acids consumed is more important than total fat in the diet. Long-term intake of saturated and trans fatty acids has been associated with insulin resistance [30]. Frequent tobacco consumers have also been found to develop early insulin resistance syndrome (IRS). Nicotine and cigarette smoking have been recognized to cause the level of stress hormone, cortisol, to rise in excess to induce insulin resistance [31]. Regarding vegetable intake, the pathway through which vegetables influence health is not well understood. However, dietary fiber in vegetables has been known to limit insulin response following meals by slowing macronutrient absorption through delayed gastric emptying [32].

Osmotic symptoms of hyperglycemia are the most strongly endorsed symptoms in DM. Symptoms of thirst; dry mouth, higher affinity for sweets; polyuria and frequency of night urination appear to be specific for hyperglycaemia in DM. Although osmotic symptoms may not appear in the early stages of impaired fasting glucose, it emerges with more prolonged or severe hyperglycaemia during the later course of the disease when islet cell damage and insulin resistance has heightened their peak [33]. Thus, in such patients, presence of osmotic symptoms can be associated with higher degree of insulin resistance.

Regarding lipid parameters, LDL cholesterol was found to be significantly correlated with HOMA-IR and HOMA %B values. Diabetes is well known for characteristic dyslipidaemia which includes elevated TG, low HDL-C, and preponderance of small dense LDL particles. Any relation between LDL particles and insulin resistance could probably be exerted through the effect of insulin on lipoprotein metabolism [34]. Accumulation of excess cholesterol in beta-cells causes lipotoxicity by decreasing expression of transcription factors required for beta-cell development and survival [35]. Roehrich et al. [36] have reported LDL-C above 6 mmol/l to induce apoptosis of beta-cells. Moderate exercise has been found to improve insulin sensitivity by reducing visceral and abdominal subcutaneous fat and maintains beta-cell function by decreasing lipotoxicity to beta-cells [37]. As for alcohol consumption, moderate alcohol intake has been associated with lower risk of T2DM whereas high and chronic intake leads to abdominal obesity and accelerates pancreatic fibrosis and beta-cell damage [38].

This study indicated TC/HDL-C ratio to be used as surrogate marker for insulin resistance and not TG/HDL-C ratio. TG/HDL-C ratio has been demonstrated to fail to predict insulin resistance in people of African-American ethnicity by Anne et al. [39]. However in a study done by Ray et al. [10] both TC/HDL-C ratio and TG/HDL-C ratios were found to be significantly correlated with HOMA-IR with excellent AUC value of 0.8 for TG/HDL-C ratio and acceptable AUC value of 0.78 for TC/HDL-C ratio. Thus, it is important to note that, although TG/HDL-C ratio has been recommended as surrogate for insulin resistance, the relation between TG and TG/HDL-C with

insulin may differ by ethnicity and hence in certain populations, using it to determine insulin resistance may not be appropriate [40,41].

Conclusion

Our study found more reduced insulin sensitivity compared to reduction in beta-cell function in new T2DM patients. Also, the degree of insulin resistance in newly diagnosed diabetic patients who have not yet developed osmotic symptoms was lesser than those who have. These findings suggest that early intensive treatment addressing insulin resistance is, in fact, the best approach to slow the development of the disease and its progression, and to possibly retard the development of chronic complications of T2DM. This necessitates the use of easy and reliable diagnostic tools such as the HOMA model for early detection of insulin resistance and beta-cell failure. Although simple, the application of HOMA model using C-peptide might be constrained in resource limited country like ours. Under such scenario, the use of cheaper and reliable surrogates of insulin resistance like the WHR and TC/HDL ratio as verified by this study should be effectively used.

Authors' Contributions

PB carried out the study, analysed the data and prepared the manuscript. PKS designed the study and supervised the work. BJ supervised the work. All authors have read and approved the manuscript.

Competing Interests

No competing interests.

Ethics Approval and Consent to Participate

Written permission to conduct the study was obtained from Institutional Review Board, Research Department, Institute of Medicine. Informed written consent was explained and obtained from the all participants.

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