



## To Study the Association of Free Insulin Levels, Obesity Markers & Lipid Profile Parameters in Newly Diagnosed Patients of Type 2 Diabetes Mellitus in Tertiary Care Centre of Kumaon Region

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### Abstract

Diabetes Mellitus is the commonest metabolic abnormality in the world. Type 2 diabetes the commonest form of diabetes constitutes nearly 90% of diabetic population in any country. Type 2 diabetes mellitus is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 diabetes mellitus. Obesity is always associated with increase in plasma triglycerides. Dyslipidemia includes hypertriglyceridemia, reduced HDL cholesterol, and increased numbers of small, dense LDL particles. The present was therefore planned to study the correlation between markers of obesity, lipid profile & insulin resistance in newly diagnosed patients of type 2 Diabetes mellitus.

**Materials & Methods:** 100 newly diagnosed patients of Type 2 DM were taken as cases. Healthy age & sex matched 100 subjects were included in control group.

**Results:** In this study 37 patients of DM were overweight and 19 were obese. All the female patients showed waist hip ratio (WHR) >0.85 and 33 males out of 43 had WHR >0.90. Mean waist circumference in diabetic cases were significantly raised when compared with healthy controls. The mean level of Triglyceride & VLDLc in diabetic cases was found to be significantly raised when compared with normal subjects. The mean level of ApoB was found to be significantly elevated in diabetics as compared with controls. In this study the levels of HbA1c & serum free insulin were also significantly raised in diabetic cases as compared to normal subjects.

**Conclusion:** This study showed that newly diagnosed diabetic patients have high incidence of obesity, insulin resistance and dyslipidemias, which predisposed them to metabolic syndrome. Significantly high levels of HbA1c in newly diagnosed DM suggests its utility as the predictor of future risk for metabolic syndrome.

**Keywords:** diabetes mellitus, dyslipidemias, obesity, insulin resistance.

### Introduction

The global epidemic of diabetes will challenge our generation to develop novel strategies to prevent and treat this lifelong condition. Every 10 seconds, two people develop diabetes and one

person dies from diabetes-related causes.<sup>(1)</sup> In 2007, 246 million people worldwide had diabetes. That number is expected to climb to 380 million by 2030 (International Diabetes Federation, 2007).<sup>(2)</sup>

In most developed countries, diabetes is the fourth or fifth leading cause of death and there is concern that it will become an epidemic in many developing and newly industrialized nations.

The global prevalence of diabetes will double in the next 30 years due to population growth, urbanization, increasing prevalence of obesity, aging, and physical inactivity (Wild S *et al.*, 2004).<sup>(3)</sup>

Diabetes can be caused by a variety of hormonal and cellular defects, which result in elevated blood glucose levels. Insulin and other hormones are released in response to rising blood glucose levels.<sup>(4)</sup>

The two broad categories of Diabetes Mellitus are Type 1 and Type 2. The unique feature of type 1 diabetes mellitus is its progressive autoimmunity resulting in complete destruction of the pancreatic beta cell. Viral triggers such as enteroviruses, coxsackie virus B, congenital rubella, cytomegalovirus, and mumps are suspected culprits (American Association of Diabetes Educators, 2006).<sup>(5)</sup>

Type 2 diabetes is a heterogeneous group of disorders that in combination result in hyperglycemia. These disorders include beta cell death, insulin resistance, excessive hepatic glucose release, and other hormone deficiencies (American Association of Clinical Endocrinologists, 2007).<sup>(6)</sup>

Type 2 diabetes mellitus is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism (Kaprio *et al.*, 1992).<sup>(7)</sup>

Obesity, particularly visceral or central (as evidenced by the hip-waist ratio), is very common in type 2 diabetes mellitus. In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets in certain individuals are unable to sustain the hyperinsulinemia state (Alvin C. Powers, 2008).<sup>(8)</sup>

insulin resistance, the decreased ability of insulin to act effectively on target tissues (especially muscle, liver, and fat), is a prominent feature of type 2 diabetes mellitus and results from a combination of genetic susceptibility and obesity (DeFronzo RA, 1988, Kahn CR, 1994).<sup>(9,10)</sup>

The obesity accompanying type 2 diabetes mellitus, particularly in a central or visceral location, is thought to be part of the pathogenic process. Obesity increases the risk for various comorbidities, and recent advances in the understanding of adipose tissue biology offer an insight into the complex pathophysiologic mechanisms.

Obesity is always associated with increase in plasma triglycerides. Dyslipidemia includes hypertriglyceridemia, reduced HDL cholesterol, and increased numbers of small, dense LDL particles (Marsh JB, 2003).<sup>(11)</sup> Other features of the dyslipidemia of abdominal adiposity include elevated very low density lipoproteins (VLDL), and reduced HDL, which are the large buoyant antiatherogenic subspecies of total HDL. In some individuals, Apo B levels may be elevated, reflecting an increase in the number of small, dense lipoprotein particles (VLDL and LDL) (McNamara J *et al.*, 1987).<sup>(12)</sup>

Overproduction of apolipoprotein B can result in lipid-induced endoplasmic reticulum stress and insulin resistance in the liver (Su Q *et al.*, 2009).<sup>(13)</sup>

Considering these facts the present study plan to correlation between the parameters of obesity, dyslipidemias and insulin resistant in newly diagnosed patients of type 2 diabetes mellitus.

### Materials and Methods

After permission from institutional ethical committee and a written informed consent, The present study was carried out in the Department of Biochemistry, in association with the Department of Medicine, Government Medical College, Haldwani, during the period of 2014 to 2015. 100 patients of newly diagnosed type 2 diabetes mellitus based on diagnostic criteria for diabetes According to American diabetes association

(ADA) were included as cases those were in age group of 20-60 years.<sup>(14)</sup> 100 age and sex matched healthy subjects were taken as controls. Patients with Gestational diabetes mellitus were excluded from the study.

All patients were subjected to detailed history and thorough clinical examination.

#### **Anthropometric measurements**

**Weight:** Body weight (in kg) was measured in light clothing and without shoes. The weight was recorded to the nearest kg.

**Height:** Height was measured without shoes with the subjects standing fully erect on a flat surface and taken to the nearest centimetre.

**Body mass index:** Body mass index was calculated by the formula- Weight in kg/ (Height in meter).<sup>2</sup>

**Waist circumference:** Waist circumference (in centimetre) was measured at midway between the costal margin & iliac crest. Waist circumference was measured at the end of normal expiration.

**Hip circumference:** Hip circumference (in centimetre) was taken as the largest circumference at the posterior extension of the buttocks (Transtrochantaric). Waist hip ratio is the waist circumference divided by the hip circumference.

Taking all aseptic precautions, about 5 ml of blood was drawn by veinpuncture from a peripheral vein, with a disposable syringe. All samples were collected in the morning after an overnight fast. Sample for fasting blood glucose, lipid profile, Apo

B, free insulin were collected in a plain vial. The blood thus collected in clean dry glass tubes was allowed to stand for 30 minutes at room temperature for the retraction of clot. This was then centrifuge at 3000 r.p.m. for 10 minutes to separate the serum. The serum was stored at 4°C in the refrigerator for analysis.

For the quantification of HbA1c, sample was collected in Ethylenediaminetetraacetic acid (EDTA) vial.

The parameters including blood sugar, lipid profile, HbA1c were analyzed in fully automated Roche/Hitachi Cobas c 501 analyzer. Serum ApoB level was analyzed in semiautomatic analyzer (MERCK Microlab 300) and estimation of serum free insulin was done in ELISA Micro plate reader (MIOS JUNIOR MERCK).

#### **Statistical Analysis**

The data were compiled and entered in MS Excel sheet and the analysis was carried out using the Statistical Package for the Social Sciences (SPSS 19.0.2) program for windows. Unpaired “t” test was used to analyze all the data for statistical significance. Correlation and regression coefficient were also calculated among relevant parameters.

#### **Results**

Table 1 shows the age and sex distribution among the studied patients. All the patients studied were of the age group 25-60 years.

**Table 1:** Distribution of age-group among the studied subjects (n=200)

Age group	Male(n=92)		Female (n=108)	
	Controls	Diabetics	Controls	Diabetes
25-35	2	2	6	6
36-45	25	20	20	22
46-55	15	14	20	24
56-60	7	7	5	5
<b>Total</b>	<b>49</b>	<b>43</b>	<b>51</b>	<b>57</b>

Table 2 shows the distribution of diabetic subjects according to their BMI. Of the 100 cases 41 subjects (17 males & 24 females) had normal BMI, 37 (16 males & 21 females) were

overweight and 19 (9 males & 10 females) were obese. Only healthy subjects with normal BMI were taken as controls

**Table 2:** Distribution of diabetic subjects according to their Body Mass Index (According to WHO criteria)

BMI (kg/m <sup>2</sup> )	Male	Female	Total
Normal (18-24)	17	24	41
Over weight (25-29)	16	21	37
Obese (>30)	9	10	19
<b>Total</b>	<b>43</b>	<b>57</b>	<b>100</b>

Table 3 shows the distribution of WHR in the studied subjects. The normal WHR ratio in male is <0.90 and female is <0.85. Among the studied

subjects 33 males had increased WHR, whereas all the females had WHR > 0.85.

**Table3:** Distribution of diabetic patients according to Waist Hip Ratio (WHR)

(The values of WHR in control males and females were <0.90 and < 0.85 respectively)

WHR	Male (n=43)		Female(n=57)		Total No
	<0.90	>0.90	<0.85	>0.85	
NO	10	33	0	57	100

Table 4 shows mean waist circumference in healthy subjects vs diabetic patients. The mean levels of waist circumference in diabetic males were significantly raised (p= 0.0001) when

compared with healthy male controls. The mean levels of waist circumference in diabetic females were also significantly raised (p= 0.0001) when compared with healthy female controls.

**Table4:** Waist Circumference in studied subjects (mean±SD).

Variables	Male with DM	Healthy Male	P value	Female with DM	Healthy Female	P value	Normal Range (cm)
Waist circumference (cm)	96.26±1.01	85.65±0.57	0.0001	90.34±0.68	75.63±0.25	0.0001	Male < 90 Female <80

Table 5 shows biochemical parameters in healthy controls vs diabetic patients. The mean level of total cholesterol in diabetic cases was mildly elevated when compared with healthy controls (230.8±32.6 vs 154±37.80; p=0.0126). The mean level of TG (256.52±24.92 vs 132±36.8; p=0.0001) and VLDLc (40.45±2.34 vs 24.10±2.15; p=0.0001) in diabetic cases was raised and highly significant when compared with healthy controls. The mean level of LDLc was raised in diabetics but statistically insignificant. The mean level of HDLc was reduced in diabetics (40.03±1.12) when compared with controls (42.51±1.51) the p value was 0.015. The mean level of ApoB was elevated in diabetics (76.89±3.92) when compared with controls (70.28±3.66) the p value was 0.032.

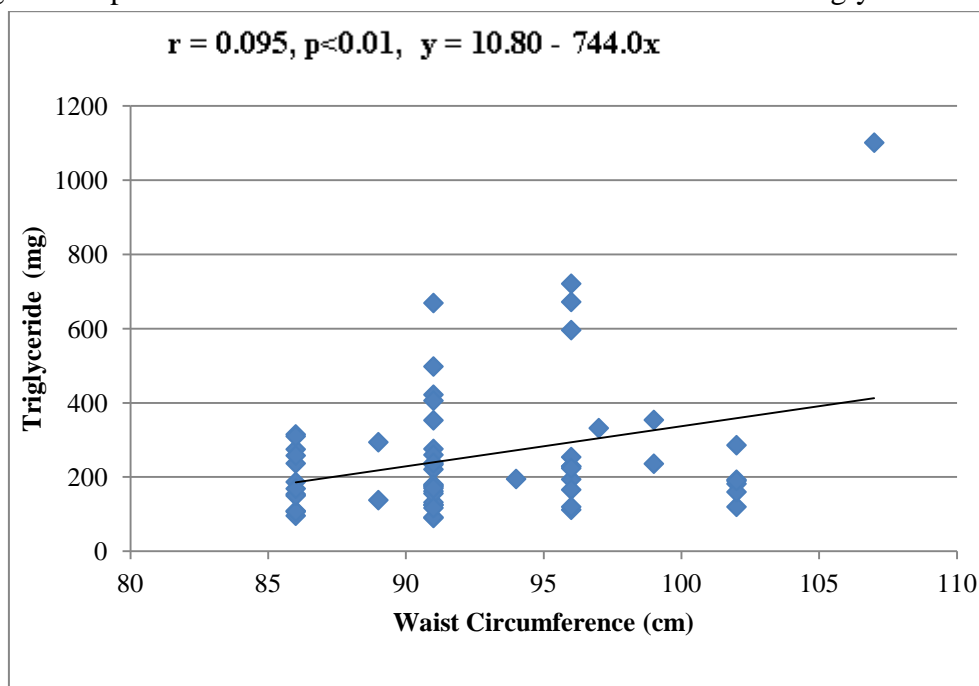
respectively p value also found to be significant (0.001)

**Table 5** Biochemical parameters studied in healthy control vs diabetic patients

Parameters	Healthy controls	Diabetic cases	P value
<b>Lipid profile (mg/dl)</b>			
Total cholesterol	154±37.80	230.8±32.6	0.0126
Triglycerides	132±36.8	256.52±24.92	0.0001
Low density lipoprotein cholesterol	83.9±27.5	108.60±35.01	0.605
Very low density lipoprotein cholesterol	24.10±2.15	40.45±2.34	0.0001
High density lipoprotein cholesterol	42.51±1.51	40.03±1.12	0.015
ApoB	70.28±3.66	76.89±3.92	0.032
<b>Blood glucose levels (mg/dl)</b>			
Fasting	86.7±9.3	137.6±42.2	0.0001
Post prandial	106±12.3	229.8±25.1	0.0001
<b>Other parameters</b>			
HbA1c (%)	4.4±0.88	7.30±0.25	0.001
Serum Free insulin (µIU/ml)	2.76±0.74	7.83±1.66	0.001

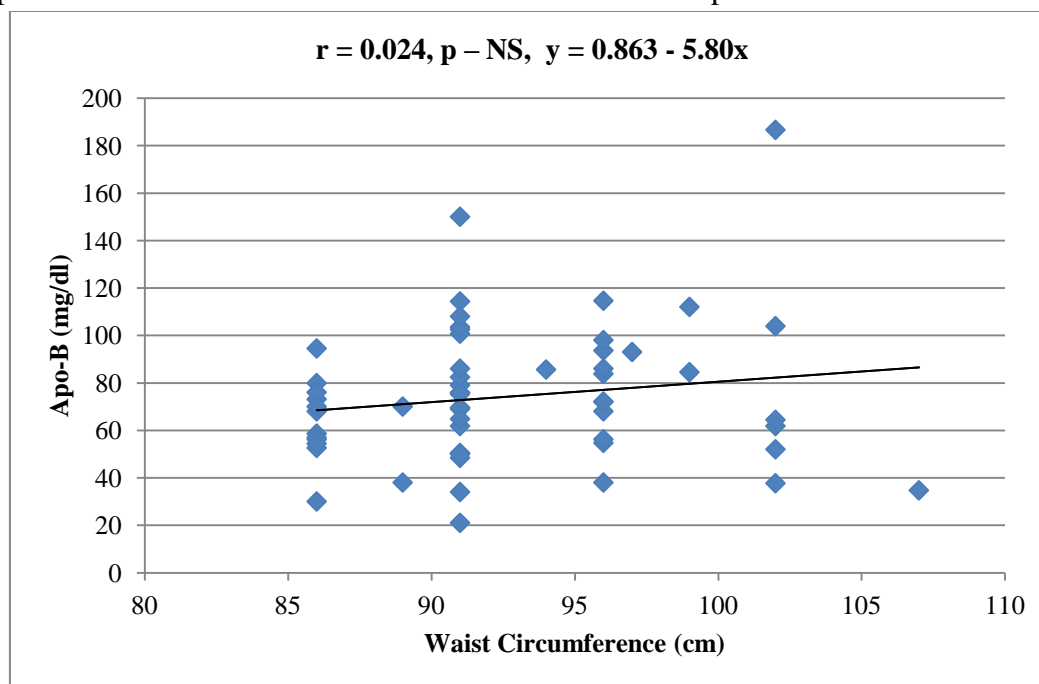
The newly diagnosed diabetic patients in this study showed higher levels of HbA1c (7.30±0.25%) as compared to healthy controls (4.4±0.88) with p value of 0.001. the mean level of serum free insulin in diabetic and healthy controls were 7.83±1.66 and 2.76±0.74

Fig 1 shows significant positive correlation between waist circumference and triglyceride levels.

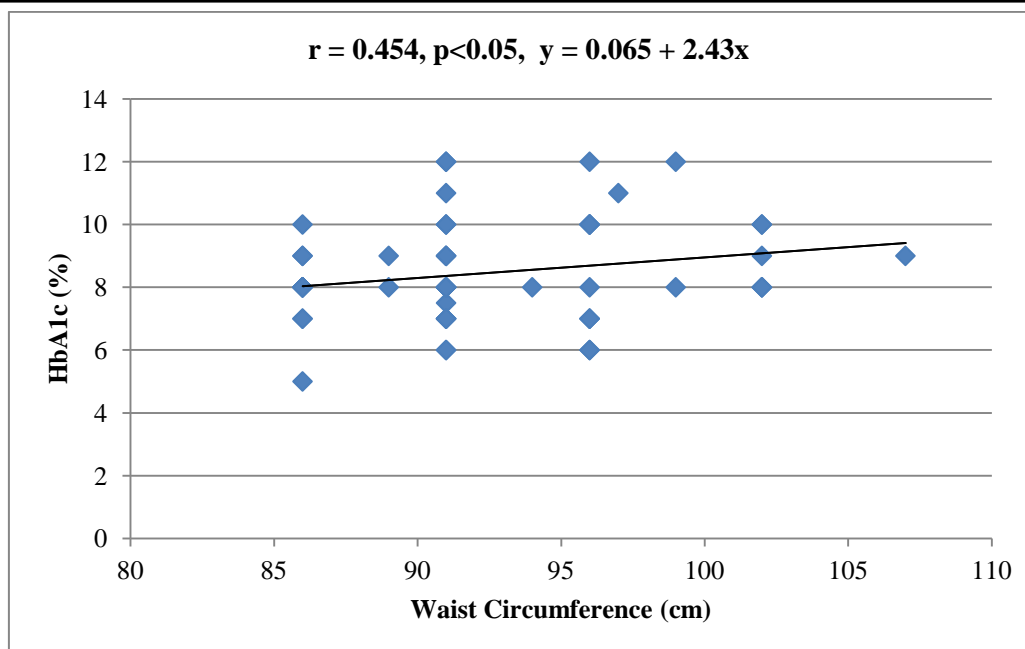


**Fig 1** Correlation between waist circumference and triglyceride levels inpatients of diabetes mellitus

Fig 2 shows positive correlation between waist circumference and ApoB levels.



**Fig 2** Correlation between waist circumference and serum Apo B levels in patients with Diabetes Mellitus



**Fig 3** Correlation between waist circumference and HbA1c levels in patients with Diabetes Mellitus

### Discussion

In this study out of 100 newly diagnosed diabetic patients, 57 were females and 43 were males. Similar observation were also shown in the study of Alshkri and Elmehdawi.<sup>(15)</sup>

Abdominal obesity characterized by high waist circumference is a stronger predictor than generalized obesity defined by elevated Body Mass Index (BMI) of subsequent development of major coronary event, vascular mortality, diabetes and metabolic syndrome. Overweight is defined as a BMI of 25-29 and obese as a BMI >30 (WHO, 2000).<sup>(16)</sup>

MA Shekhar *et al.*, 2005 in their study in Mysore on urban patients found the BMI to be 23.9 in males and 25.3 in females.<sup>(17)</sup> In the study of Alshkri and Elmehdawi, 2008 out of 99 patients, 61 were females and 38 were males of all patients, 74.4% were obese and obesity was significantly more frequent among females ( $p < 0.001$ ). Mean BMI was 33.6 kg/m.<sup>2</sup> (18) In our study according to BMI, obesity was predominant in females as compared to males, which is comparable with the above studies.

Waist-hip ratio (i.e. the waist circumference divided by the hip circumference) was suggested as an additional measure of body fat distribution. In our study WHR was increased in all the

females studied ( $>0.85$ ) while 33% of males had increased WHR ( $> 0.90$ ). Various studies have shown that WHR is an important cardiovascular risk factor and greater levels are associated with multiple risk factors. Gupta R *et al.*, 2003 reported that WHR  $>0.9$  in men and  $>0.8$  in women is associated with a significant increase in multiple risk factors.

Dyslipidemia, characterised as an increased free fatty acid, triglyceride, small dense LDLc and low HDLc levels is widely accepted risk factor for coronary heart disease. In our study serum TG and VLDL levels were significantly elevated in patients of diabetes mellitus as compared to healthy subjects. Whereas no significant difference was found in levels of total cholesterol, LDLc and HDLc.

Study by VS Prasad *et al.*, 2015, observed high levels of serum triglyceride in diabetes Mellitus.<sup>(19)</sup> Huges *et al.*, 2011, showed that relative risk of Myocardial infarction correlates directly with increased triglyceride and inversely with HDL-c levels in both Caucasians and Asians Indians.<sup>(20)</sup> The combination of abdominal obesity and low HDL was also reported as the most common combination among Chinese type-2 diabetics (Lee YJ, Tsai JC, 2002).<sup>(21)</sup>

Apo B is one of the component found in chylomicron, VLDL, intermediate density lipoprotein and LDL. The association of Apo B with diabetes has potential role as a subclinical inflammatory agent (Faraj M *et al.*, 2006).<sup>(22)</sup> Apo B is a good surrogate measure of increased LDL particle numbers in people with insulin resistance (Williams K *et al.*, 2003), and small LDL particle number was best correlated with Apo B and triglycerides and HDL-cholesterol) in the Framingham Heart study (Kathiresan S *et al.*, 2006).<sup>(23,24)</sup>

The present study shows Serum Apo B levels was significantly elevated in patients of diabetes Mellitus. Similarly Lim *et al.*, 2011, found serum Apo B to be higher in diabetic patients.<sup>(25)</sup> Ryoo *et al.*, 2013, conducted a cohort study which followed up 25,193 healthy Korean males of diabetes mellitus without metabolic syndrome for 5 years, and reported that Apo B was a predictive factor for metabolic syndrome.<sup>(26)</sup>

Insulin resistance has been defined as a defect in insulin action that results in hyperinsulinemia, necessary to maintain euglycaemia. Many people with insulin resistance have high levels of blood glucose and high levels of insulin circulating in their blood at the same time.<sup>(27)</sup>

In our study serum free insulin level was significantly elevated ( $p < 0.005$ ) in patients of diabetes mellitus as compared to those normal controls. Our study is supported by David E *et al.*, 2002, that over 95 percent of men who had the hyperglycemia had hyperinsulinemia.<sup>(28)</sup>

HbA1c is the most commonly measured parameter for long term monitoring of diabetes mellitus. The level of HbA1c has been widely accepted as an indicator of mean daily blood glucose concentration over the preceding 8-12 weeks. In our study HbA1c levels were significantly elevated ( $p < 0.01$ ) in patients of diabetes mellitus as compared to normal controls.<sup>(29)</sup>

These results suggest that HbA1c may be a surrogate marker not only of future diabetes, but also of CVD. Although there are many studies

which report the utility of HbA1c in predicting CVD and diabetes. Jasmin S *et al.*, 2008, observed that metabolic syndrome group showed significantly higher glucose, HbA1c levels and waist circumference.<sup>(30)</sup>

### Conclusion

This study concludes that in patients with diabetes mellitus there is high incidence of obesity and increased waist hip ratio. The serum parameters in present study also showed that there is significant elevation of atherogenic lipids, HbA1c and insulin resistance in diabetics. There is significant positive correlation between parameters of obesity and dyslipidemia. These data provide evidence that newly diagnosed patients of DM are at future risk of metabolic syndrome. Thus physician treating type 2 diabetics should consider metabolic syndrome with greater emphasis. Life style modification like weight loss, exercise of  $\geq 30$  min per day in take of high dietary fibers can decrease the incidence of morbidity & mortality associated with complication diabetes.

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