Original article:

Efficacy of Fasting glucose-Leptin ratio as a risk marker for development of long term diabetic complications

[1]Srivatsan R, [2]Suresh S

[1]Assistant Professor, [2]Associate Professor

Department(s) and institution(s) :

1 - Department of Biochemistry, Government Tiruvannamalai Medical College & Hospital

2 - Department of Biochemistry, Government Tiruvannamalai Medical College & Hospital

Corresponding Author: Dr Suresh S, Associate Professor **,** Department of Biochemistry **,** Government Tiruvannamalai Medical College & Hospital , Tiruvannamalai,Tamilnadu 606 604 , India

ABSTRACT

Background: To evaluate the usefulness of glucose-leptin ratio in identifying long term diabetic complications development.

Methods: In this case control study, we took 75 non obese diabetic patients with clinical evidence of diabetic complications and remaining 75 non obese diabetics with no clinical evidence of diabetic complications. Routine blood and urine biochemistry, HbA1C levels and leptin levels were measured. Fasting glucose-leptin ratio was calculated for all the patients.

Results: Leptin levels get significantly decreased among patients with diabetic complications when compared to those without them irrespective of sex of the patient. GL ratio seems to be significantly increased in the presence of diabetic complication. More than 50% of both male and female patients above their respective GL ratio cutoffs of 13.5 and 10.5, were having one or more long term diabetic complications.

Conclusion: In the absence of obesity, leptin levels may relatively decline during the latter stages of diabetes mellitus when complications start showing up irrespective of the age or sex of the patient or the type of complications. GL ratio could possibly be used as a risk marker for development of diabetic complications.

Key words: Diabetes mellitus, Leptin, Microvascular complications, acrovascular complications, Glucose-leptin ratio

Introduction:

Leptin, a protein encoded by *'ob'* obese gene, is a hormone secreted by adipocytes that plays a significant role in regulating food intake, energy expenditure and neuroendocrine function. Tissue adiposity along with gender are the main determinants of leptin gene expression and release [1]. Animal studies suggest that there is an inverse relationship between leptin levels and insulin secretion [2]. It is also being suggested that the association between plasma leptin and diabetes may be a manifestation of underlying leptin resistance mediated obesity [3]. Numerous studies have indicated a positive association with leptin levels and development of diabetic complications. However, majority of them have not eliminated the single main confounding factor 'obesity' while finding the relationship. In this context, we tried to evaluate the usefulness of glucose-leptin ratio in identifying long term diabetic complications development.

Materials and Methods:

This study was conducted for a period of one year from Jan 2018 to Dec 2018 at a rural tertiary care Government hospital in south India after getting approval from the Institutional Scientific and Ethical committee. The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its latter amendments. Our case control study included 150 clinically confirmed diabetic patients between the age group of 35 to 55 years. Out of those, 75 diabetic patients with clinical evidence of diabetic microvascular or/ and macrovascular complications were regarded as cases and remaining 75 diabetics with no clinical evidence of diabetic complications were considered as controls. All patients included in our study were non-obese diabetic patients [Body Mass Index (BMI) 18.5-22.9 kg/m^2] of both the sexes. Patients with clinical evidence of diabetic nephropathy, diabetic retinopathy & diabetic neuropathy/ diabetic foot ulcers were included as patients having microvascular diabetic complications. Likewise, patients who had only diabetic related cardiovascular complications or any vessel related complications were included and classified as patients having macrovascular complications. Underweight/ overweight or obese individuals (BMI <18.5 and ≥23.0 kg/m^2), patients having extremes of age (< 35 years and >55 years), patients not clinically diagnosed with diabetes, patients having any chronic co-existing disease conditions along with diabetes, patients on insulin therapy, diabetic patients with previous history of any form of renal, cardiovascular, ophthalmic, peripheral vascular disease complications developed even before the onset of diabetes and critically ill patients were excluded from our study.

After explaining the nature of the study, written consent was obtained from all subjects before collecting blood sample. Using standard measures of height and weight, BMI was measured using Quetelet's index [BMI = weight (kilograms)/height ($metre^2$)]. Classification of patients according to their BMI was done according to the cut-offs set for the Asian population by WHO [4]. The study population of diabetic patients were stratified into two groups based on the presence or absence of diabetic complication by physical examination and investigation. Fundus examination was done by an ophthalmologist to rule out the presence of retinopathy. Serum urea, creatinine and urine microalbumin-creatinine ratio were done to know the presence of nephropathy. ECG/ Echo were taken to identify cardiovascular complication. Physical examination for foot ulcers and nerve conduction studies were done to confirm the presence of neuropathy.

All biochemical analysis in serum and urine were carried out in Dimension RxL max Integrated Chemistry system from Siemens healthineers. HbA1C measurement was carried out by ion exchange resin method using Glycated Hemoglobin kit from Biosystem**.** Serum leptin values were evaluated using sandwich ELISA method using leptin-ELISA kit from DIA.

Statistical analysis

Data was analyzed using SPSS software 16.0 version for windows. All values showing parametric distribution were presented as mean ± standard deviation (Mean ± SD). Non-parametric distribution values were presented as median ± interquartile range (IQR) between 25^{th} and 75^{th} percentile. Comparison between cases and controls were carried out by independent sample t-test in case of parametric distribution and Mann Whitney test in case of non-parametric distribution. Receiver Operating Characteristic (ROC) curve was constructed to check for diagnostic accuracy of GL ratio in assessing the diabetic complications. Binary logistic regression analysis was performed to ascertain the impact of independent variables on likelihood of having diabetic complications. A 'p' value of less than 0.05 was considered statistically significant.

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Results:

Out of the 150 clinically confirmed non-obese diabetic patients, 83 (55%) were males and 67 (45%) females. Mean age was 47±6 years for males and 46±6 years for females. Among male patients, 40 (48%) were having no clinically evident microvascular or macrovascular complications. 43 (52%) male patients showed evidence of long term diabetic complications. Corresponding numbers for diabetic female patients were 35 (52%) and 32 (48%) respectively. Of the 43 male patients with diabetic complications, 30 (70%) were evidently having either microvascular or macrovascular complications. Remaining 13 (30%) male patients showed presence of both microvascular as well as macrovascular complications together. Likewise, among 32 female patients showing complications, 20 (62%) had either microvascular or macrovascular complications and the rest 12 (38%) had both microvascular as well as macrovascular complications together. We regarded patients with HbA1C value ≤ 7.0 as having good glycemic control. 7 (8%) of male patients and 14 (21%) of female patients were having a good glycemic control. Mean fasting glucose-leptin ratio (GL ratio) was for male and female diabetic patients respectively. The demographics and patient's general characteristics were summarized in Table 1.

ROC curves were constructed for male and female diabetic patients to check the efficacy of GL ratio in predicting diabetic complications. It showed area under the curve (AUC) of 0.872 (0.798-0.946) for male and 0.820 (0.718-0.922) for female diabetics for long term diabetic complications. A cutoff GL ratio of 13.5 for male was found to show a maximal sensitivity and specificity (81.4% sensitive, 70.0% specific) for identifying diabetic complication among male case. It had 74.5% positive predictive value and 77.8% negative predictive value with a positive and negative likelihood ratios of 2.71 and 0.27 respectively. The corresponding cut-off among female patients was found to be 10.5 having 78.8% sensitivity, 73.5% specificity, and 74.3% positive predictive value, 78.1% negative predictive value with a positive and negative likelihood ratios of 2.98 and 0.29 respectively (Table 2; Fig 1 and Fig 2).

Table 3 shows the distribution of patients above the calculated GL ratio cut-off for male and female diabetics with respect to different conditions of patient. As evident from the table, more than 50% of both male and female patients above their respective GL ratio cutoffswere having one or more long term diabetic complications/ were having a poor glycemic control and were diabetics for more than five years.

Regression analysis was made to assess the impact of age and biochemical analysis for glycemic control, renal function, lipid profile and leptin on male and female patients having microvascular and macrovascular complications. Male patients having both above GL ratio cut-off of 13.5 showed a positive likelihood with increase in age, HbA1C, serum urea, creatinine, triglycerides, HDL cholesterol and urine microalbumin-creatinine ratio, while total cholesterol, showed a negative likelihood when compared with male patients below GL ratio cut-off of 13.5. The corresponding positive and negative odds, among female patients were similar except for urea and HDL cholesterol. However barring HbA1c levels for both sexes and urine microalbumin creatinine ratio exclusively among female none of the other parameters were showing statistical significance. The model could explain only 32.2% of variance for male patients, correctly classifying 74.7% of cases and 60.4% variance for female patients with 76.1% cases classified correctly (table 4).

Discussion:

The main aim of this study was to understand the efficacy of Glucose-leptin ratio as a risk marker for development of long term diabetic complications. We try to eliminate obesity factor, the significant confounder for variations in leptin levels, for our study by selecting diabetic patients having a normal body-mass index (18.5-22.9 kg/m^2).Also confounder bias due to gender related alteration of leptin values was eliminated by running separate statistics for

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both the sexes. Age related confounder in leptin was taken care by selecting all the patients between a narrow age group of 35 to 55 years for our study. Our results revealed that leptin levels get significantly decreased among patients with diabetic complications when compared to those without them irrespective of sex of the patient. GL ratio seems to be significantly increased in the presence of diabetic complication. More than 50% of both male and female patients above their respective GL ratio cutoffs of 13.5 and 10.5, were having one or more long term diabetic complications/ were having a poor glycemic control and were diabetics for more than five years. It does not seem to get influenced by age, glycemic status, renal function, lipid profile and urine microalbumin-creatinine ratio among both sexes, especially after development of long term microvascular or macrovascular complications.

Numerous studies have indicated that gender plays a significant role in the variation of leptin levels. Luukkaa et al. [5] in their study on 269 elderly and young non-diabetic men found that administration of testosterone negatively influenced the secretion of leptin levels, which reverted back to its pretreatment levels once testosterone administration was stopped. Isidori et al. [6] in their study have explained the inverse relation between leptin, fat mass and total testosterone as the binding of testosterone to androgen-binding receptors on the adipocytes increases lipolysis or has a direct suppressive effect on *ob* gene expression. At the same time Lavoie et al. [7] in their study found an increase in serum leptin level when there is administration of short term physiological estrogen replacement. Widjaja et al. [8] found the leptin levels were higher in diabetic females than diabetic males after adjustment for BMI and ethnic groups. Our study had a mean leptin level significantly lower in men compared to women irrespective of the presence or absence of diabetic complications. The difference in leptin level may possibly be due to gender difference in sex hormone levels. Gender difference in distribution of body fat may probably not be a factor for leptin difference as our selected group of cases and controls were having normal BMI.

Our study has found a decrease in leptin levels when the diabetic patients had microangiopathy and macroangiopathy irrespective of the gender, when compared to those without complications. Insulin stimulates the production of leptin from adipose tissue, and leptin, in-turn inhibit insulin secretion, thereby reducing the adipose tissue formation which is called adipo-insulinar axis [9]. Thus, during early stages of type II diabetes when the insulin resistance is minimal with marginal hyperinsulinemia, there will be relative increase in the levels of leptin, although it is independent on the amount of adipose tissue [10]. However, during the late stages of uncontrolled diabetes mellitus, when there is high likelihood of development of complications, there would be more severe insulin resistance and reduction in beta cell function of pancreas and much reduced insulin secretion. This in turn will reduce the stimulation of leptin production.

There are varying results regarding the association of leptin levels with diabetic complications. Some studies even regard leptin to have a protective role in preventing the development of complications, conversely increasing the risk of complications when their level comes down. Naito et al. [11] in their study of leptin action in LepTg Akita mice have concluded that leptin relieved systemic oxidative stress significantly and this decrease in oxidative stress may dampen the development of chronic diabetic complications. Ebihara et al. [12] in their prospective controlled trial had found that long term leptin replacement therapy in patients with generalized lipodystrophy had ameliorated macro and microalbuminuria and showed no deterioration of neuropathy and retinopathy in those patients. It also improved insulin sensitivity and insulin secretion dramatically. Hanai et al.
in their observational cohort study on 668 patients concluded that both low and high serum levels of leptin were risk factors for decline in kidney function, and leptin administration in patients with generalized lipodystrophy had improved albuminuria and other metabolic parameters. Conversely, Sari et al. [14] in their case control study on 157 T2DM patients and 46 healthy controls concluded that leptin levels may not be strongly associated with

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complications of type II diabetes mellitus. Ren J. [15] in his review had concluded in his study that elevated plasma leptin level plays a specific role in the intricate cascade of cardiovascular events, triggering the development of cardiovascular disease. Leptin had a pressor effect and increased the symphathetic tone both centrally and peripherally increasing the symphathetic outflow to kidneys, adipose tissue, skeleton and adrenal medulla along with stimulation of endothelial nitric oxide and upregulate endothelin-I production and accumulation of ROS in human umbilical vein endothelial cells thereby worsening the angiopathies according to Yamagishi et al. [16] and Quehenberger et al. [17].

While the variation in leptin levels with or without presence of complications is still debatable, it could be regarded that the leptin decrease in our study has to do mainly with low adiposity because all the patients selected were having normal BMI. By the time, the diabetic complications start developing, there will be absolute loss of beta cell of pancreas leading to drastic reduction in insulin secretion, coupled with relatively low adiposity (normal BMI) possibly reducing the secretion of leptin further during the time of diabetic complications, in our study. Of note is that variations in the odds ratio for numerous parameters including leptin levels with regard to development of diabetic complications between both the sexes may possibly be due to variation in the percentage fat and sex hormones between male and female subjects though there isn't a significant difference in BMI. Further large interventional studies may be warranted to confirm this. Considering the topical matter of exploring the variation in leptin levels, it would had been more reasonable had we measured the actual levels of percentage body fat and insulin levels for both the sexes, which we feel as the limitation of our study.

Conclusion:

Our data identified that in the absence of obesity, leptin levels may relatively decline during the latter stages of diabetes mellitus when complications start showing up irrespective of the age or sex of the patient or the type of complications. GL ratio could possibly be used as a risk marker for development of diabetic complications.

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Table 1: AUC of ROC curve and cut off values of Glucose-Leptin ratio with their corresponding to their maximum sensitivity and specificity in identifying diabetic complications

	AUC	95% CI	Cut-off value	Sensitivity%	Specificity%	PPV%	NPV%	LR+	LR-
GL Ratio (For Male)	0.872	0.798 to 0.946	13.5	81.4	70.0	74.5	77.8	2.71	0.27
GL Ratio (For Female)	0.820	0.718 to 0.922	10.5	78.8	73.5	74.3	78.1	2.98	0.29

AUC – Area Under ROC Curve; PPV – Positive Predictive Value; NPV – Negative Predictive Value; LR+ -> Positive Likelihood Ratio; LR- -> Negative Likelihood Ratio;

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Variables	Male (n=83)			Female (n=67)		
	Estimates	Odds ratio for having GL ratio above cut-off of 13.5 (95% CI)	*p* value	Estimates	Odds ratio for having GL ratio above cut-off of 10.5 (95% CI)	*p* value
Age	0.015	1.02 (0.93-1.11)	0.740	0.070	1.07 (0.93-1.23)	0.327
HbA1C	1.341	3.82 (1.74-8.42)	0.001*	2.445	11.53 (3.36-39.54)	<0.001*
Serum urea	0.028	1.03 (0.94-1.12)	0.538	-0.083	0.92 (0.82-1.04)	0.184
Urine microalbumin-creatine ratio	0.004	1.00 (0.99-1.01)	0.155	0.010	1.01 (1.00-1.02)	0.038*
Cholesterol (Total)	-0.004	0.99 (0.98-1.01)	0.526	0.004	1.00 (0.98-1.02)	0.728
Triglycerides	0.002	1.00 (0.99-1.01)	0.682	0.002	1.00 (0.99-1.02)	0.816
Cholesterol	0.010	1.01 (0.92-1.11)	0.844	-0.122	0.89 (0.76-1.03)	0.105

Table 2: Logistic Regression– Impact of age and different biochemical analytes for male and female diabetic patients above their respective GL ratio cut-off

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Figure 1: ROC Curve - GL ratio Vs Presence of one or more long-term diabetic complications among male diabetic patients

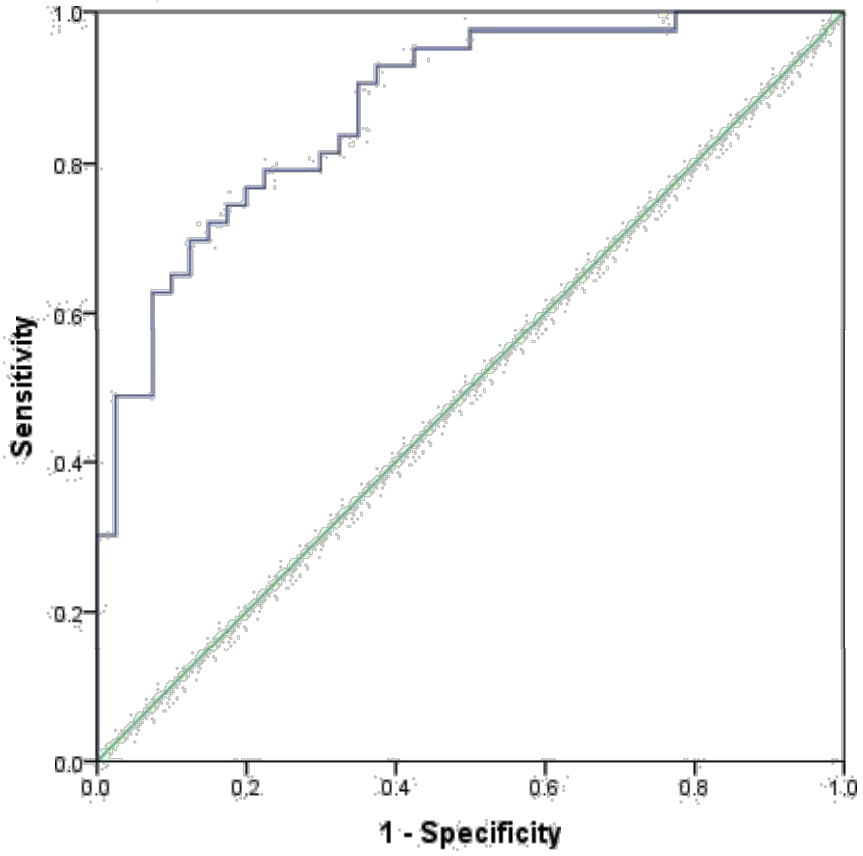

Figure 2: GL ratio Vs Presence of one or more long term diabetic complications among female diabetic patients