Determination of Nonalcoholic Fatty Liver Disease in Patients with Pre-Impaired Glucose Tolerance

Valerie Ann U. Valdez, M.D.*; Leilani B. Mercado-Asis, M.D., PhD, MPH*; Amy A. Lopez, M.D.*; Erick S. Mendoza, M.D.*; Katherine Jane G. Barredo, M.D.**; Jose D. Sollano, M.D.**; Abigail M. Milo, M.D***; Mario T. Milo, M.D.***

Abstract

Introduction: Pre-impaired glucose tolerance (pre-IGT) or compensated hyperinsulinemia, is defined as normal glucose, and elevated insulin two hours after a 75-gram oral glucose load. It is characteristic of the early stages of diabetes mellitus (DM), where beta cells compensate for insulin resistance by increasing insulin secretion to maintain normoglycemia. With continuing beta cell failure, insulin secretion eventually fails, leading to the progression to diabetes. Nonalcoholic fatty liver disease (NAFLD), a common feature of insulin resistance, is found in 50-75% and 42-55% of DM and pre-diabetes patients. We determined if NAFLD was present in patients with pre-IGT.

Method: A study on the determination of NAFLD – diagnosed by liver ultrasound in pre-IGT patients at a university hospital. Descriptive statistics, Chi square test of independence, 2x2 Fischer Exact test, Z test of difference in proportion, were used for statistical analysis with a p-value set at 0.05α. IBMSPSS ver 21 was used as software.

Results: The mean age of 22 patients was 29.95 years, with average BMI of 25.73 kg/m^2; 77.3% were female. Average lipid panels were within optimal limits; kidney and liver functions were normal. The mean insulin level was 58.36 ulU/mL. NAFLD was identified in eight of the subjects.

Conclusion: Although pre-IGT is a subclinical phase in the diabetes spectrum, 36% already have NAFLD. This prevalence was lower compared to diabetics and pre-diabetics, but higher compared to the general population. There was a noticeable trend of increasing insulin levels with increasing severity of fatty liver.

Keywords: pre-IGT; compensatory hyperinsulinism; NAFLD;

Introduction

Diabetes mellitus (DM) type 2 is a progressive disease characterized by beta cell dysfunction and decreasing insulin sensitivity. In its early stages, the beta cell compensates for insulin resistance by increasing insulin secretion to maintain blood glucose within normal levels; this has been labeled as "pre-IGT" (pre-impaired glucose tolerance) or compensated hyperinsulinemia by Matawaran et al.[1] in 2009. The local study identified the presence of pre-IGT in 42-52% of individuals who were high risk to develop DM.[1,2] Since it was a relatively new concept, research attempts have been made to explore the different characteristics of this new metabolic profile. In a manuscript by Nisce and Mercado-Asis (2011), they attempted to develop a clinical scoring to identify pre-IGT state, however, it has not shown to be helpful, signifying that pre-IGT is subclinical in nature.

*Section of Endocrinology, Diabetes & Metabolism, Department of Medicine, University of Santo Tomas Hospital, Manila
**Section of Gastroenterology, Department of Medicine, University of Santo Tomas Hospital, Manila
***Ultrasound Section, Department of Radiology, University of Santo Tomas Hospital, Manila, Philippines

Corresponding author: Valerie Ann U. Valdez, M.D., University of Santo Tomas Hospital, Manila, Philippines
E-mail: vauvaldez@yahoo.com

With continuing beta cell failure, increasing insulin levels can no longer compensate for the insulin resistance and insulin secretion eventually fails, leading to the progression to type 2 DM.

Nonalcoholic fatty liver disease (NAFLD) is a common feature of insulin resistance, and is characterized pathologically by a spectrum encompassing simple steatosis, non-alcoholic steatohepatitis and cirrhosis. The prevalence of NAFLD has been reported to be in the range of 10-24%[3] in the general population. Among those with type 2 diabetes mellitus, the prevalence was as high as 50-75%.[4,5,6] Recent studies have shown that even patients with IGT or pre-diabetes have high prevalence of NAFLD (42-55%).[5,6] As the degree of insulin resistance worsens, so does the severity of fatty liver disease; conversely, insulin resistance predicts nonalcoholic hepatic steatosis.[5]

Although the gold standard in the diagnosis and staging of NAFLD is liver biopsy, ultrasonography is one modality used to identify fat in the liver in the early, preclinical form of the disease[7]. It has the advantage of being readily available, noninvasive, requires only a short time to perform and with immediate results, making it a good screening method of NAFLD in a general population. A scoring system using abdominal ultrasonography was developed by Hamaguchi

Table I. Hamaguchi scoring system for fatty liver[8]

Bright liver and hepatorenal echo contrast	
0	Bright liver and hepatorenal echo contrast were negative
1	Either bright liver was positive or hepatorenal echo contrast was positive
2	Bright liver was mild, and hepatorenal echo contrast was positive
3	Bright liver was severe, and hepatorenal echo contrast was positive
Deep attenaution	
0	Deep attenuation was negative
1	Visualization of the diagphragm was obscure, but an observer could distinguish the diaphragm
2	An observer could not distinguish the diaphragm
Vessel blurring	
0	Vessel burning was negative
1	The borders of intrahepatic vessels were unclear and the lumen of intrahepatic vessels was narrowed
Sum score of A, B, and C, if score of A is more than 1; Total score is 0, if score of A is 0	

et al.[8] in 2007 to diagnose histologically proven NAFLD. It had high sensitivity (91.2 - 92.6%) and specificity (100%), which was actually more accurate compared to previous reports. The scoring system was noted to have high reliability, with within-observer and between-observer reliability of 0.95 (95% CI 0.93-0.97, P<0.001); and it utilizes a simple criterion, both necessary for a screening method (Table I).

There is much to be discovered about the unique characteristics of pre-IGT. When this group was first identified, it was observed that patients with pre-IGT had higher BMI values compared to the normal and pre-diabetic group, however statistical significance was not established [1]. The same held true for insulin levels two hours after an oral glucose load. In a separate study on pre-IGT patients, they observed that glycosylated hemoglobin (HbA1c), second hour blood glucose and second hour insulin levels were significantly higher in the pre-IGT group compared to those patients with normal oral glucose tolerance test (OGTT)[2]. A positive correlation was demonstrated between the HbA1c and second hour glucose levels but not with the second hour insulin levels. In knowing the characteristics of this earliest stage of the diabetes spectrum, multiple approaches may be devised to prevent the development of overt diabetes, and its long-term complications. Since NAFLD is associated with a constellation of clinical problems that arise from insulin resistance, we hypothesize that in the pre-IGT state, which is a subclinical state of glycemic abnormality, NAFLD may not be apparent.

The general objective of this study is to determine if nonalcoholic fatty liver disease is present in patients with pre-IGT.

The specific objectives include 1) to describe the clinical profile of pre-IGT patients with and without NAFLD; 2) to identify other risk factors present in pre-IGT patients that may contribute to the development of fatty liver; 3) to determine if there is a relationship between fatty liver and the patients' clinical and metabolic profile; and 4) to determine if a relationship exists between fatty liver and post-glucose load insulin levels.

Methods

This is a single-center study to determine the prevalence of NAFLD in pre-IGT patients, conducted in a university hospital, and duly approved by the local Institutional Review Board. Purposive, nonprobability sampling was utilized since the target population was limited, and the disease of interest is relatively new.

Patient Profile

A total of 22 patients who were diagnosed with pre-IGT in a private specialty clinic were recruited to participate in the study. All participants have read, understood and signed an informed consent prior to study entry, and the research conducted in accordance to the ethical principles in the Declaration of Helsinki. A diagnosis of pre-IGT was established using a 75-gram OGTT, and defined as having normal second hour glucose (less than 140 mg/dL), and elevated insulin levels (greater than 30 uIU/mL)[1]. Individuals who underwent testing were those who were high risk to develop diabetes mellitus: e.g. family history of diabetes in first-degree relatives, previous history of gestational diabetes, polycystic ovary syndrome and obesity.

Inclusion And Exclusion Criteria

Patients eligible to be enrolled in the study may be male or female, aged 18 years old and above, with a diagnosis of pre-IGT. Participating patients who had a history of chronic

glucocorticoid intake or were previously diagnosed to have dyslipidemia or any known liver disease were automatically excluded. Likewise, those who had significant alcohol intake (defined as more than 20 grams/day) or a history of intake of lipid-lowering agents were also not included.

Data Gathering

An initial interview was conducted to obtain baseline patient information. All data were encoded in a data collection sheet. Patient identity was kept confidential, identified with a code known only to the author. Only the researchers had full access to patient information and data. Demographic data included age and sex; anthropometric measurements done were weight (kg), height (cm), and waist circumference (cm), with subsequent calculation of the body mass index (BMI) in kg/m^2. Pertinent laboratory data included lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL)), liver enzymes and insulin level on diagnosis.

Procedure

Participants were instructed to have an overnight fast of four to six hours prior to undergoing a liver ultrasound. The procedure was done using the Siemens s3000 machine at the Ultrasound Department of the institution where the study was conducted. To decrease inter-observer variability, the procedure was done by a single technician and interpreted by the same radiologists, all blinded by the characteristics of the subjects.

A diagnosis of nonalcoholic fatty liver was made using ultrasonographic results and the Hamaguchi Scoring System[8]. The imaging parameters utilized were hepatorenal echo contrast, bright liver, deep attenuation and vessel blurring – as outlined in Table I. A score of ≥ 2 was needed to fulfill the diagnosis NAFLD. The presence of fatty liver was then further classified into mild, moderate and severe. Mild was defined as having mild increase in the fine echoes in the hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders; moderate as a diffuse increase in fine echoes with mild impaired visualization of the intrahepatic vessels and diaphragm; and severe as marked increase in fine echoes with non-visualization of the intrahepatic vessel borders, diaphragm and posterior portion of the right lobe of the liver.[8,9]

Statistical Analysis

All data were encoded in MSEXCEL 2013. Descriptive statistics was used to determine measures of central tendencies such as mean and standard deviations. Categorical data were presented in frequencies and percentages, while continuous data were indicated using mean and standard deviations. To compare rates, Chi square test of independence, 2x2 Fischer Exact test, and Z test of difference in proportion were used. Any associated p-values lesser than 0.05 alpha were considered statistically significant. To ensure accuracy of computation, IBMSPSS version 21 and SAS 9.1 were used as aids in processing the data.

Results

Clinical Profile

A total of 22 subjects participated in this study, composed mostly of females (77.3%); mean age was 29.95 years, with an age range of 23 to 48 years. Table II shows the baseline demographic data and anthropometric characteristics of subjects. The average body mass index (BMI) of all patients was 25.73 ± 4.12 kg/m^2, with 77.2% of the patients belonging to the overweight and obese category. The mean waist circumference of 84.39 ± 8.76 cm, signified the presence of abdominal obesity (defined as waist circumference of ≥ 80 cm for women and ≥90 cm for men.

Table II. Baseline Characteristics Of Patients

Patient Charactersitics	Descriptive	
Demographic		
Age (years), mean ± SD	29.95	±7.29
Sex, f%		
Male	5	22.70%
Female	17	77.30%
Anthropometric		
BMI[a] (kg/m^2), mean ± SD	25.73	±4.12
Normal (18.5-22.9), f%	5	22.70%
Overweight (23-24.9), f%	3	13.60%
Obese 1 (25-29.9), f%	12	54.50%
Obese 2 (≥ 30), f%	2	9.10%
Waist (cm), mean ± SD	84.39	±8.76
Male	90	±6.74
Female	82.74	±16.27

[a] BMI- body mass index; World Health Organization-Asia Pacific criteria[11]

Metabolic Profile

The average baseline lipid profiles of patients in Table III were within normal to near optimal levels[11]. Liver function, was likewise normal; with average SGPT of 22.44 U/L and SGOT of 17.28 U/L. Mean insulin levels, two hours after a 75-gram oral glucose load was 58.36 uU/mL.

Ultrasonographic Profile And Correlation With Patient Profile

Out of the 22 patients, eight or 36% were found to have fatty liver based on ultrasonography criteria. Of the eight patients, six had mild and two had moderate fatty liver; but none had severe. Table IV presents a cross tabulation of fatty liver against the different clinical and metabolic profile present in the patient. It is evident that patients who had fatty liver had a lower mean age. On the average, patients with and without fatty liver had abdominal obesity according to sex-specific definitions. Among male patients, those who had normal liver were observed to have greater waist circumference than those who had fatty liver. This was conversely true among female subjects, wherein those who had fatty liver had more abdominal adiposity. A similar trend of having higher values for those without fatty liver was observed for HDL levels.

Table III. Metabolic Profile Of Patients

Metabolic Profile	Mean	Standard Deviation
Lipid profile (mg/dL)		
Total cholesterol	178.17	28.46
Triglyceride	74.26	31.72
HDL[a]	61.2	20.78
LDL[b]	101.98	24.95
Liver function test (U/L)		
SGPT[c]	22.44	10.66
SGOT[d]	17.28	3.68
75-Gram OGTT[e]		
2nd hour insulin (uU/mL)	58.36	26.61

[a]HDL-high density lipoprotein; [b]LDL-low density lipoprotein; [c]SGPT-serum glutamate-pyruvate transaminase; [d]SGOT-serum glutamic oxaloacetic transaminase; [e]OGTT-oral glucose tolerance test

The BMI, total cholesterol, LDL, and liver functions were similar in both groups. Patients with fatty liver were noted to have higher triglyceride levels compared to those without.

At baseline, pre-IGT patients already have elevated second hour insulin levels, since it is a requisite for its diagnosis. It was observed that patients who had fatty liver had higher insulin levels. On further analysis, there was a trend of increasing insulin levels as the severity (mild, moderate, severe) of fatty liver progressed. However, analysis of the mean values showed that the differences of values among those patients with and without fatty liver were not statistically significant.

Table IV. Clinical and Metabolic Profile of Patients With and Without Fatty Liver

	Fatty Liver		
	Negative n=14	Positive n=8	
Clinical Profiles	Mean (Range)	Mean (Range)	p-value
Age (Years)	30.77 (23-48)	28.63 (23-40)	0.527
Waist (cm)			
Male	92.67 (85.5-97)	86.00 (81-91)	0.494
Female	80.86 (60-95.5)	86.18 (81.5-94.5)	0.060
BMI[a] (kg/m^2)	25.08 (19.3-36.8)	26.87 (23-31.2)	0.341
Total cholesterol (mg/dL)	181.61 (138-237)	172.16 (160-196)	0.467
Triglyceride (mg/dL)	68.44 (45-149)	84.45 (43-165)	0.265
HDL[b] (mg/dL)	66.62 (45-139)	51.72 (45-60)	0.107
LDL[c] (mg/dL)	101.23 (57-148)	103.30 (86-129)	0.857
SGPT[d] (U/L)	22.87 (9-53)	21.70 (10-39)	0.812
SGOT[e] (U/L)	16.30 (10-21)	18.50 (11-24)	0.217
Insulin (uU/mL)	52.48 (31.4-112.5)	68.65 (31.2-106.4)	0.176

[a]BMI – body mass index; [b]HDL-high density lipoprotein; [c]LDL-low density lipoprotein; [d]SGPT-serum glutamate-pyruvate transaminase; [e]SGOT-serum glutamic oxaloacetic transaminase

Discussion

Hyperinsulinemia

Increasing evidence show that by the time glucose tolerance or fasting glucose levels become impaired, pancreatic beta cell destruction may have already occurred. At the same time, patients who have glucose tolerance or those with completely normal OGTT findings may have elevated insulin to maintain glucose levels within normal levels – a state of compensated hyperinsulinemia that equates to insulin resistance[1]. There is a high prevalence and rapidly increasing trends of hyperinsulinemia from 25% to 34.8% according to NHANES[12], with the most dramatic changes occurring in the 20- to 39-year-old group for both sexes. This finding is consistent with data showing the greatest increase in obesity prevalence among people aged

18-29 years.[13] Both statistical data validate hyperinsulinemia and obesity characterizing the pre-IGT patients of the same age group.

NAFLD and Insulin Resistance

Fatty acids (FFAs) in the liver come from three different sources: dietary fat, adipocytes (lipolysis) and de novo hepatic lipogenesis. Excess caloric intake in the diet can result in obesity and associated insulin resistance. This contributes to the development of fatty liver by impairing the ability of insulin to suppress lipolysis, which leads to increased delivery of FFAs to the liver. The increased FFAs may then induce hepatic insulin resistance. As to whether insulin resistance in NAFLD is the cause or the consequence, is still not clear.[4]

NAFLD is strongly associated with reduced whole-body insulin insensitivity, documented by 45-50% reduction of glucose disposal; impaired ability of insulin to suppress endogenous glucose production (hepatic insulin resistance); and a defect in insulin suppression of free fatty acid (adipose insulin resistance). All these findings suggest the possibility of insulin resistance as an intrinsic defect in NAFLD[4].

Associations of insulin resistance and NAFLD have been established in patients with pre-diabetes and type 2 diabetes. NAFLD has a high prevalence among type 2 diabetic patients, while slightly lower in pre-diabetics as described above. Since pre-IGT precedes the development of the latter, the presence of NAFLD is expected to be minimal at this point. In this study, NAFLD was already present in 36% of individuals with pre-IGT. It is lower than the prevalence reported in patients with pre-diabetes and diabetes but higher compared to that of the general population. The trend of increasing prevalence across worsening beta-cell function proves that pre-IGT is the first phase metabolic dysfunction in the spectrum of glucose homeostasis. At the same time, this data further supports the important role of insulin resistance in the pathogenesis of fatty liver.

Among the conditions associated with insulin resistance, obesity is the most common factor associated with NAFLD[4]. Both of these risk factors lead to increased free fatty acid delivery to the liver or impaired lipolysis, coupled with increased de novo lipogenesis, leading to the development of fatty liver disease.[14] As shown in Table II, the BMI of the subjects are widely distributed across the different classifications, however more than 60% are clustered under the obese category. A cross-section of BMI and fatty liver in Table V illustrates that those with normal BMI did not have fatty liver. At the same time, being obese, did not necessarily equate to having NAFLD. All those who were found to have fatty liver were overweight/obese. However, the p-value of 0.226 signified that an association of fatty liver with increasing BMI levels in this subset of patients could not be established.

Table V. Body Mass Index Classification of Patients With and Without Fatty Liver

	Fatty Liver		
Clinical Profile	Negative n=14	Positive n=8	p-value
BMI[a] (kg/m^2) f%			
Normal (18.5-22.9)	5 (36%)	0 (0)	0.226
Overweight / Obese (≥23)	9 (64%)	8 (100%)	

[a]BMI- body mass index; World Health Organization-Asia Pacific criteria[11]

The lipid profiles of patients in the study are mostly within optimal levels[11] to eliminate dyslipidemia as a cause of the development of fatty liver. However, this did not protect these patients from developing fatty liver, as noted. Due to the p-value across all lipid panels, there is not enough statistical evidence to ascertain their relationship.

Focusing on the association of insulin levels with fatty liver, it is important to bear in mind that pre-IGT patients already have elevated insulin levels. Although the calculated p-values did not allow the establishment of a statistically significant association, those with fatty liver had higher insulin levels, and there was a trend of increasing insulin levels with the progression of the severity of fatty liver disease (e.g. insulin level of 60 uU/mL for mild disease and 88 uU/mL for moderate disease on further analysis). Increasing sample size in future studies may be able to eventually help determine if such a relationship exists or if a cut-off value for insulin level could be identified that would be predictive for developing mild, moderate and severe fatty liver.

In a study by Viswanathan, the prevalence of obesity, hypertension and dyslipidemia were significantly higher in diabetic subjects with NAFLD.[15] Microvascular and macrovascular complications were also significantly higher in diabetic subjects with NAFLD. Whether these complications are already present in pre-IGT with NAFLD remains to be investigated. What is significant is that as early as the pre-IGT state, appropriate lifestyle, and possibly therapeutic inventions, must be given to patients to prevent the progression of NAFLD as well as prevent other complications associated with insulin resistance. But despite the presence of risk factors like hyperinsulinemia and obesity in patients with pre-IGT, a statistically significant association could not be established with the development of fatty liver disease. The plausible reason that it did not reach statistical level of significance would be the small sample size. Since this subset of patients has only been recently described, there is a paucity of such population. And since it is not a routine practice to check for insulin levels after an oral glucose load in high-risk individuals, only a limited number

of patients will be able to undergo screening and eventually be diagnosed.

It has been shown that pre-IGT or compensated hyperinsulinemia, represents the earliest stage along the spectrum of developing diabetes mellitus, thus highlighting the subclinical nature of this condition. And since they are more likely to develop cardiovascular disease, essential hypertension, and nonalcoholic fatty liver disease[13], preventing the development of these long-term complications, would also alleviate the great financial burden – to the individual and the society - that is associated with it. We recommend early detection of pre-IGT patients among high-risk individuals. By increasing awareness among these individuals, this presents an opportunity to delay the onset of morbidity related to hyperinsulinemia. On a more practical standpoint, knowing this high prevalence of insulin resistance among Filipinos may offer a window for health care practitioners to refine present practice to include the determination of insulin levels, to be able to identify these patients early on in the disease process.

Conclusion

In conclusion, the incidence of nonalcoholic fatty liver disease in patients with pre-IGT is 36%, which is lower compared to diabetics and pre-diabetics, but higher compared to the general population. Despite the presence of hyperinsulinemia and obesity, which are known risk factors for the development of fatty liver, more sufficient evidence is needed to support its association with pre-IGT. An important observation made, however, was that of increasing insulin levels with increasing disease severity. Perhaps, further studies with adjusted and larger samples could detect the differences among the values of insulin level among those with fatty liver.

Acknowledgements

The authors would like to thank all patients who consented and participated in this research endeavor. To Jesse Batara, for his immense contribution in the statistical analysis of data. And to all colleagues who took part in the study, thank you.

References

1. **Matawaran B, Asis L;** Comparison of Pancreatic Insulin Response to Hyperglycemia Among Filipino Subjects of Various Glycemic Status. Phil J Int Med, 47: 25, 2009.
2. **Torres-Salvador P, Asis L**; Glycosylated Hemoglobin and Second Hour Glucose Level after Oral Glucose Tolerance Test (OGTT) were Significantly Higher in Individuals in the Pre-Impaired Glucose Tolerance (Pre-IGT) State versus those with Normal Oral Glucose Tolerance Test and Normoinsulinemic. Endocr Rev, 33:3, 2012.
3. **Parekh S, Anania F;** Abnormal Lipid And Glucose Metabolism in Obesity: Implications For Nonalcoholic Fatty Liver Disease. Gastroenterology, 132: 2191, 2007.
4. **Utzschneider K, Kahn S;** REVIEW: The Role of Insulin Resistance in Nonalcoholic Fatty Liver Disease. J Clin Endocrinol Metab, 91(12): 4753-61, 2006.
5. **Vernon G, Baranova A, Younossi ZM;** Systematic Review: The Epidemiology and Natural History of Non-alcoholic Fatty Liver Disease and Non-alcoholic steatohepatitis in Adults. Aliment Pharmacol Ther, 34: 274, 2011.
6. **Mohan V, Farooq S, Deepa M,** Ravikumar R, Pitchumoni C; Prevalence Of Nonalcoholic Fatty Liver Disease in Urban South Indians in relation to different grades of Glucose Intolerance and Metabolic Syndrome. Diabetes Res Clin Pract, 84(1): 94, 2009.
7. **Kennedy G;** Non-alcoholic Fatty Liver Disease: Can Ultrasound Assist in Early Diagnosis of Prediabetes and Delay Progression to Type 2 Diabetes Mellitus. QUT, 2009.
8. **Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T**; The Severity of Ultrasonographic Findings in Nonalcoholic Fatty Liver Disease Reflects the Metabolic Syndrome and Visceral Fat Accumulation. Am J Gastroenterol, 102: 2708, 2007.
9. **Gerstenmaier J, Gibson R**; Ultrasound In Chronic Liver Disease. Insights Imaging, 5(4): 441, 2014.
10. **World Health Organization**, Western Pacific Region, International Association for the Study of Obesity, International Obesity Task Force. The Asia-Pacific Perspective: Redefining Obesity and its Treatment. February 2000.
11. **Third Report of the National Cholesterol Education Program (NCEP)** Expert Panel on Detection, Evaluation nd Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Circulation, 106:3143, 2002.
12. **National Health and Nutrition Examination Survey (NHANES)** Hepatic Steatosis Ultrasound Images Assessment Procedures Manual. Massachusetts Medical Society. N Engl J Med, 346:1221, 2002.
13. **Li C, Ford E, McGuire L, Mokdad A**, Little R, Reaven G; Trends in Hyperinsulinemia Among Nondiabetic Adults in the U.S.. Diabetes Care, 29(11): 2396, 2006.
14. **Salt, W;** Nonalcoholic Fatty Liver Disease (NAFLD): A Comprehensive Review. J Insur Med, 36:27, 2004.
15. **Viswanathan V, Kadiri M, Medimpudi S, Kumpatla S**; Association of Non-alcoholic fatty liver disease with diabetic microvascular and macrovascular complications in South Indian Diabetic Subjects. Int J Diabetes Dev Ctries, 30(4): 208, 2010.