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RESEARCH ARTICLE

Ultrasonographic Evaluation of Severity of Hepatic Steatosis in Type2 Diabetic Patients Treated with Oral Hypoglycemic Drug Metformin-Correlation with Serum C-Peptide and Triglyceride Level

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Abstract

This cross sectional study was carried out in the department of radiology and imaging, BIRDEM in collaboration with department of Cell and Molecular Biology, Research division, BIRDEM from June 2006 to May 2007 with an aim to investigate the role of transabdominal sonography to diagnose fatty change in liver in patients suffering from type2 diabetes mellitus treated with metformin and to correlate the grading of fatty change to c-peptide and triglyceride levels. The study was conducted in 92 type2 diabetic patients from both sexes between 44 years to 68 years of age. In present study, hepatic steatosis at ultrasonography was considered as grade-0 (normal liver echogenicity without any fatty infiltration); grade-1 (minimal diffuse increase in hepatic echogenicity, normal visualization of diaphragm and intrahepatic vessel borders); grade-2 (moderate diffuse increase in hepatic echogenicity, slightly impaired visualization of intrahepatic vessels and diaphragm) and grade-3 (marked increase in liver echogenicity; poor or nonvisualization of the hepatic vessels and diaphragm). In this study, out of 92 subjects, fatty infiltration in liver (increased hepatic echogenicity) of different grade was detected in 72 subjects by ultrasonography. Significant age differences were found between four groups. Fasting c-peptide and triglyceride showed significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity. Waist hip ratio (WHR) showed significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity. Body mass index (BMI) also showed significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity. Waist hip ratio (WHR), serum triglyceride (Tg), grade of liver echogenicity and body mass index (BMI) had significantly positively associated with fasting c-peptide (FCP) level. It was also observed that type 2 diabetes mellitus is a predisposing factor for the development of fatty liver. At the same time, it was seen that increased body mass index (BMI) plays an important role in its development. Waist hip ratio (WHR) has an association with the severity of fatty change in the liver. It was also observed that there is a linear correlation between the severity of liver echogenicity and fasting c-peptide and triglyceride level.

Keywords: Ultrasonographic, Hepatic Steatosis, Type2 Diabetic, Serum C-Peptide, Triglyceride Level.

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1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia (High blood sugar) and other symptoms like polyurea, polydipsia, and polyphagia. Weight loss may also occur. World Health. Organization (WHO) has recognized three main forms of diabetes: Type1, Type2 and gestational diabetes. Type 1 diabetes is usually due to autoimmune destruction of pancreatic beta cells, which produce insulin. Type2 diabetes is characterized by tissue-wide insulin resistance and varies widely; it sometimes progresses to loss of beta cell functions. Gestational diabetes is similar to type2 diabetes, in that it involves insulin resistance; the hormones of pregnancy cause insulin resistance in those women genetically predisposed to developing this condition and it typically resolves with delivery. In 2006, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double [1-3]. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will likely be found by 2030 [4-6]. Insulin resistance is characterized by Hyperinsulinaemia. Insulin is initially synthesized in the form of proinsulin. In this form the alpha and beta chains of active insulin are linked by a third polypeptide chain called the connecting peptide or c-peptide. For every molecule of insulin in the blood, there is one of c-peptide. The c-peptide test can be used to help assess if high blood glucose is due to reduced insulin production or to reduced glucose intake by the cells. The ectopic accumulation of fat in the liver has been strongly associated with insulin resistance, an almost universal finding in NAFLD. The prevalence of NAFLD is high in conditions associated with insulin resistance such as obesity, type2 diabetes mellitus, dyslipidemia and the metabolic syndrome. In obese population NAFLD may affect up to 75% of subjects [7]. NAFLD is very common in type2 diabetes population with between 50 and 75% of subjects demonstrating fat in the liver by ultrasound. NAFLD is strongly associated with both hepatic and adipose tissue insulin resistance as well as reduced whole body insulin sensitivity. NAFLD is characterized by remarkable reduction of insulin sensitivity, with decreased insulin effect on both glucose and lipid metabolism [7]. Serum c-peptide level in type2 diabetes is either normal or reduced than normal. Metformin is a drug which decreases in insulin resistance and it was observed that metformin increases insulin mediated glucose utilization in peripheral tissues and has an anti-lipolytic effect that lowers serum free fatty acid concentration. In that study metformin was found to cause a decrease in serum insulin and c-peptide concentrations by producing a significant improvement in insulin action. Sonographically NAFLD is characterized by increased echogenicity of liver. Normal hepatic echogenicity is more similar to renal parenchyma than to the peripheral fat echogenicity. Increased hepatic echogenicity is more similar to peripheral fat than to the renal parenchymal echogenicity [8]. Whatever the causes of NAFLD, hepatic sonographic findings are graded depending on the amount of fat infiltrated in the liver are as Grade 0, Grade 1, Grade 2 and Grade 3 [9].On the other hand, liver biopsy is the gold standard to diagnose non-alcoholic fatty liver disease, which is an invasive procedure and most of the patients and clinicians want to avoid biopsy. So ultrasonography remains as the cheapest, easily available, reliable and non-invasive diagnostic modality for the evaluation of fatty liver disease. The study was conducted to observe the association between liver echogenicity and c-peptide and triglyceride level in type2 diabetic patients who were being treated by oral hypoglycemic drugmetformin. At the same time, multiple regression analysis was performed to show the correlation of c-peptide level with waist circumference, triglyceride level, waist-hip ratio (WHR) and liver echogenicity.

2. Methods

This cross sectional study was conducted on consecutively selected 96 subjects, who were suffering from type2 diabetes mellitus and were being treated with oral hypoglycemic drug - metformin. Patients were selected from outpatient department and Health education department of BIRDEM. An informed consent was obtained from each patient. Of them 60 were male and 36 were female and aged between 44 and 68 years. After reviewing the clinical history, clinical examination of each patient was performed. Anthropometry (body height, body weight, hip circumference and waist circumference) of all subjects were measured. Detailed sociodemographic data, family history of diseases and medical history were taken. Among 96 subjects, 4 were excluded from the study as they were being treated with insulin along with metformin. The remaining 92 subjects had undergone sonographic examination of liver after

proper preparation and grading of hepatic steatosis was done as grade 0, 1, 2 and 3. All 92 subjects, of whom grading of hepatic steatosis were done, had undergone for biochemical examinations, for the estimation of fasting c-peptide, fasting blood glucose and Trigleceride. Sampies for biochemical parameters were collected between 7-30 and 9-30 AM after an overnight fast. Finally 92 subjects were included in the statistical analysis.

2.1. Inclusion Criteria

The patients who had fulfilled the following criteria were selected for this study

1. Patients who were suffering from type-2 diabetes for more than 02 years.
2. Patients who were being treated by oral hypoglycemic drug metformin.

2.2. Exclusion Criteria

1. Patients who were suffering from type 1 diabetes.
2. Patients who were being treated with insulin along with metformin.
3. Patients who had given history of regular ingestion of alcohol.
4. Diabetic patients who were suffering from other chronic and debilitating disease.
5. Patients who had given history of viral hepatitis and other liver disease.

2.3. Sample Size

A total of 92 patients were included in the study.

2.4. Sampling Method

Consecutive nonrandomized purposive sampling was done in type 2 diabetic patients who were being treated by oral hypoglycemic drug metformin, were selected in BIRDEM outpatient department and Health Education Department.

3. Preparation of Patients for Ultrasonography and Biochemical Analysis

All patients selected at OPD and Health Education Department was given at least three days preparation- For three days- restriction from meat, vegetables and fat rich foods. For two days- Tab. Ultra carbon, 2 tabs thrice daily and Tab. Lenexa, 2 tabs at bedtime. Patients were requested to fast overnight and to report at around 7-30 A.M in the department of Radiology and Imaging for sonographic examination. At ultrasonography, grading of liver echogenicity was done and selected patients were accompanied to the department of 'Cell and Molecular Biology', Research division, for biochemical investigations.

4. Scanning Technique

Patients were scanned in supine position after an overnight fast. Ultrasonogram was performed with one of the departmental sonography equipments (Sonoline, Siemens. Toshiba. Acuson 128xP/14) using 3.5 MHz. curvilinear probes. At first coupling agent was applied liberally over the right upper abdomen and then over the rest of the abdomen as examination proceeded. The correct gain setting allowed the diaphragm to be clearly seen. The liver when normal was appeared homogeneous throughout its depth. Normal tubular structures were seen clearly (the portal veins with bright edges and hepatic veins without bright edges). Hepatic artery radicals and intrahepatic biliary channels were not seen unless dilated. During scanning all patients were asked to take a deep breath and hold it in. Scanning was done in sagittal, transverse and oblique planes, including scans through intercostal and subcostal spaces. Scanning was done with a slow rocking movement of the transducer in all planes to obtain the best visualization of the whole liver.

5. Interpretation

Fatty infiltration in the liver was graded according to the following criteria:

1. Grade 0: Normal liver echogenicity without any fatty infiltration.
2. Grade 1: Minimal diffuse increase in hepatic echogenicity, normal visualization of diaphragm and intrahepatic vessel borders.
3. Grade 2: Moderate diffuse increase in hepatic echogenicity, slightly impaired visualization of intrahepatic vessels and diaphragm.
4. Grade 3: Marked increase in liver echogenicity; poor penetration of the posterior segment of the right lobe of the liver and poor or nonvisualization of the hepatic vessels and diaphragm.

6. Blood Sampling Technique

05 ml. of venous blood was collected from each patient following all aseptic precaution from the anticubital vein using disposable plastic syringe.

Blood samples were allowed to clot for 10 minutes and then centrifuged for 10 minutes at rate of 3000 rpm at 4°C. Serum samples were preserved at -70°C until biochemical analysis. Separated plasma was divided into two aliquots. The first aliquot was kept at 4°C and was used within three days to measure plasma glucose and Trigleceride. The second aliquot was frozen at -70° C until assay for C-peptide concentration. Plasma was not thrown out until the assay is completed.

7. Analytical Methods of Lab Analysis

The fasting plasma glucose concentration was measured automatically by glucose oxidase method. C-peptide was measured by Chemiluluminence based ELISA technique. Serum triglyceride was measured by enzymatic colorimetric method.

8. Estimation of Blood Glucose

Serum glucose estimation was done by glucose oxidase method using Auto analyzer, AMS (Analyzer Medical system, Rome, Italy).

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The aldehyde group of glucose is oxidized by glucose oxidase to give glucoronic acid and hydrogen peroxide. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4 aminophenazone to form a red-violet quinoneimine dye as indicator.

9. Procedure

More than 250 µl samples was given in the sample cup and reagent is taken in the reagent container. These are placed in respective space in reaction cell of IMMULITE. The instrument was programmed for the estimation of serum insulin and the IMMULITE systems automates the entire assay process in the following way:

Sample and reagent was automatically pipetted into the Test U, which is then incubated at 37° c with intermittent agitation. Following incubation, the test unit in spun at high speed about its vertical axis. Reaction fluid is forced up and completely and completely captured into the stump chamber.

A series of washes removes unbound materials efficiently from the bead and inner tube.

The bound level was than come in contact with chemiluminescent substrate, which is added to the test unit. Light emission was detected with a high sensitivity photon counter or photomultiplier tube and printed report, for each sample was generated by the system's computer. Concentration of c-peptide in sample is expressed as ng/ml.

9. Data Collection

After informing the aims and objectives, along with its procedure, risks and benefits, the informed written consent was taken. Data were collected from primary source starting from clinical history, diabetes case history book and per abdominal sonographical examinations. Data were also collected from specific serum biochemical examinations. The data were recorded in a predesigned data collection sheet (Proforma) as described in appendix-1.

10. Statistical Analysis of Data

All the relevant collected data were compiled on a master chart first. Percentages were calculated to find out the proportion of the findings. The data were expressed as the mean ± SE. Statistical method includes ANOVA test, Pearson's correlation coefficient test, and multiple regression analysis. P value of 0.05 was considered statistically significant. All statistical analysis was performed with software Statistical Package for Social Science (SPSS) 12.0 for windows (SPSS, Inc. Chicago, USA). The results were presented in tables, figures and diagrams.

11. Results

The study included 92 subjects out of which 20(21.7%) was grade 0, 33(35.9%) grade 1, 28(30.4%) grade 2 and 11(12.0%) was grade 3. All grades were divided into three age groups. The mean age of the grade 0 was 52.3±1.0 years (mean ± SE) with age range 44-60 years, mean age of the grade 1 was 55.7±1.0 years (mean ± SE) with age range 46- 68 years, mean age of the grade 2 was 58.5±1.1 years (mean ± SE) with age range 48-68 years and mean age of the grade 3 was 58.3±1.4 years (mean ± SE) with age range 50-65 years, It was observed that among the grade 0, highest percentage were (70.0%) in the age range of 50-59 years followed by 25.0% in the age range of <50 years and the lowest (5.0%) in the age range 60-69 years. Among the grade 1, highest percentage were 51.5% in the age range of 50-69 years followed by 30.3% in the age range 60- 69 years and the lowest (18.2%) in the age range <50 years. Among the grade 2, highest percentage were 46.4% in the age range of 50- 69 years & 60-69 years and the lowest (7.1%) in

the age range <50 years. Among the grade 3, highest percentage were 54.5% in the age range of 60-69 years followed by 45.5% in the age range 50-59 years and none was found in the age range <50 years. Analysis revealed that statistically significant ($p<0.05$) age difference was found among four groups in ANOVA test. The age distribution of 92 subjects was shown in Table I.

Table I. *Age distribution of the study subjects according to grading of hepatic echogenicity (n=92)*

Age in year	Grade 0 (n=20)		Grade 1 (n=33)		Grade 2 (n=28)		Grade 3 (n=11)	
	n	%	n	%	n	%	n	%
<50	5	25.0	6	18.2	2	7.1	0	0.0
50-59	14	70.0	17	51.3	13	46.4	5	45.5
60-69	1	5.0	10	30.3	13	46.4	6	54.5
Mean ±SE	52.3±1.0		55.7±1.0		58.5±1.1		58.3±1.4	
Range	(44 60)		(46 68)		(48 68)		(50 65)	

F value = 5.683, df (between group=3, within group 88), p=0.001

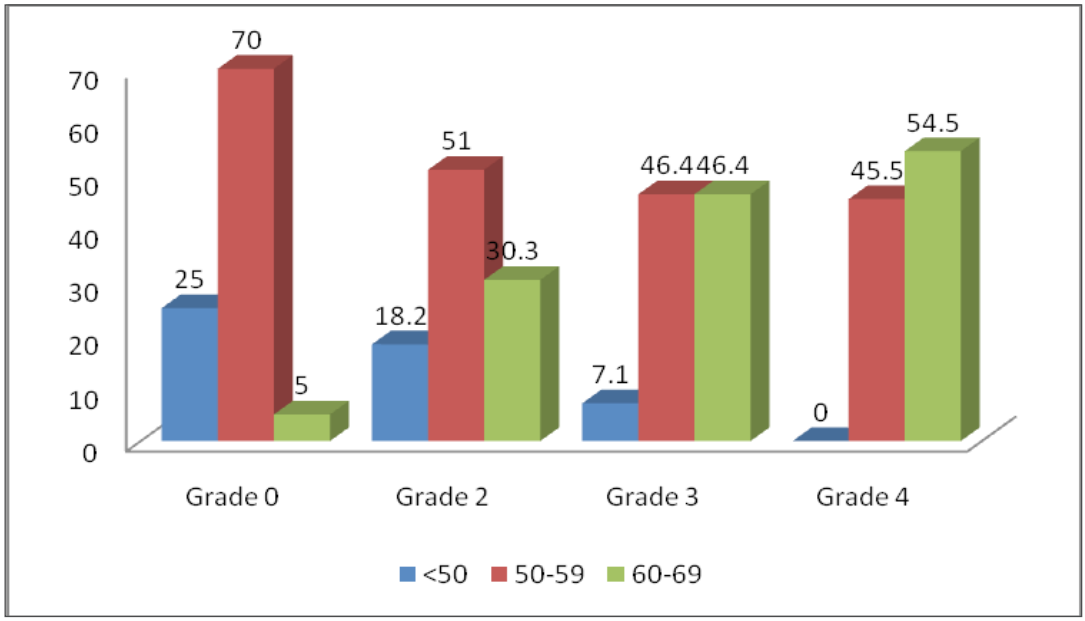

Figure 1. *Bar Diagram showing age distribution of the study subjects*

The study included 92 subjects and the mean fasting C-peptide was 1.59±0.09 ng/ml (mean ± SE) ranged 1.15 - 2.5 ng/ml in grade 0, 2.40±0.12 ng/ml (mean ± SE) ranged 1.15 - 3.75 ng/ml in grade 1, 3.59±0.10 ng/ml (mean ± SE) ranged 2.85 - 4.50 ng/ml in grade 2, 4.88±0.18 ng/ml (mean ± SE) ranged 3.80 - 5.55 ng/ml in grade 3. The mean of fasting C-peptide showed significant difference between grade 0, grade 1, grade 2 and grade 3 in ANOVA test. The result was shown in Table II.

Table 2. *Distribution of fasting C-peptide of the study subjects (n=92)*

FCP	Mean (ng/ml)	±SE	Min (ng/ml)	Max (ng/ml)
Grade 0	1.59	±0.09	1.15	2.50
Grade 1	2.40	±0.12	1.15	3.75
Grade 2	3.59	±0.10	2.85	4.50
Grade 3	4.88	±0.18	3.80	5.55

F value = 99.26, df (between group=3, within group 88), p=0.001

The mean waist hip ratio (WHR) was 0.81±0.003 (mean ± SE) ranged 0.79 0.83 in grade 0, 0.84±0.002 (mean ± SE) ranged 0.81 - 0.85 in grade 1, 0.86±0.002 (mean ± SE) ranged 0.84 - 0.89 in grade 2, 0.92±0.005 (mean ± SE) ranged 0.89 - 0.95 in grade 3. The mean of waist hip ratio showed significant difference between grade 0, grade 1, grade 2 and grade 3 in ANOVA test. The result was shown in Table III.

Table 3. *Distribution of waist hip ratio (WHR) of the study subjects (n=92)*

WHR	Mean	±SE	Min	Max
Grade 0	0.809	±0.003	0.79	0.83
Grade 1	0.84	±0.002	0.81	0.85
Grade 2	0.86	±0.002	0.84	0.89
Grade 3	0.92	±0.005	0.89	0.95

F value = 217.85, df (between group=3, within group 88), p=0.001

The mean triglyceride (Tg) was 144.9±2.67 mg/dl (mean ± SE) ranged 125.0 - 162.0 mg/ml in grade 0, 181.60±4.80 mg/dl (mean ± SE) ranged 145.0 - 230.0 mg/dl in grade 1, 279.9±8.23 mg/dl (mean ± SE) ranged 185.0 340.0 mg/dl in grade 2, 321.2±10.55 mg/dl (mean ± SE) ranged 260.0 - 360.0 mg/dl in grade 3. The mean of triglyceride (Tg) showed significant difference between grade 0, grade 1, grade 2 and grade 3 in ANOVA test. The result was shown in Table IV.

Table 4. *Distribution of triglyceride (Tg) of the study subjects (n=92)*

TG	Mean (ng/dl)	±SE	Min (ng/dl)	Max (ng/dl)
Grade 0	144.9	±2.67	125.0	162.0
Grade 1	181.6	±4.80	145.0	230.0
Grade 2	279.9	±8.23	185.0	340.0
Grade 3	321.2	±10.55	260.0	360.0

F value = 121.49, df (between group=3, within group 88), p=0.001

The mean body mass index (BMI) was 24.73±0.35 kg/m² (mean ± SE) ranged 22.4-28.6 kg/m² in grade 0, 26.67±0.24 kg/m² (mean ± SE) ranged 23.50 - 29.40 kg/m² in grade 1, 28.29±0.30 kg/m² (mean ± SE) ranged 25.20 - 30.80 kg/m² in grade 2, 29.85±0.38 kg/m² (mean ± SE) ranged 28.20 - 32.30 kg/m² in grade 3. The mean of body mass index (BMI) showed significant difference between grade 0, grade 1, grade 2 and grade 3 in ANOVA test. The result was shown in Table V.

Table 5. *Distribution of body mass index (BMI) of the study subjects (n=92)*

BMI	Mean (kg/m²)	±SE	Min (kg/m2)	Max (kg/m2)
Grade 0	24.73	±0.35	22.4	28.6
Grade 1	26.67	±0.24	23.50	29.40
Grade 2	28.29	±0.30	25.20	30.80
Grade 3	29.85	±0.38	28.20	32.30

F value = 36.87, df (between group=3, within group 88), p=0.001

Table VI shows the multiple regression analysis and observed Fasting C-peptide (FCP) had a significant association. Waist hip ratio (WHR), triglyceride (Tg), grade and body mass index (BMI) had significantly positively associated with higher fasting C-peptide (FCP).

Table 6. *Multiple regression analysis of FCP (n=92).*

Variables	Slope	±SE	t value	P value	95% CI	
WHR	28.32	±2.04	13.91	0.001	24.28	32.37
Tg	0.01	±0.00	16.14	0.001	0.01	0.02
Grade	1.09	±0.06	16.96	0.001	0.96	1.22
BMI	0.34	±0.04	7.74	0.001	0.26	0.43

Fasting C-peptide (FCP) of 92 cases was expressed in ng/ml and waist hip ratio (WHR) was measured. Significant positive correlations were found between fasting C-peptide (FCP) and waist hip ratio (WHR). The values of Pearson's correlation coefficient were 0.829 in fasting C-peptide (FCP). This was significant ($p<0.05$). Therefore, there was linear positive correlation between fasting C-peptide (FCP) and waist hip ratio (WHR) in the study population (Figure 2).

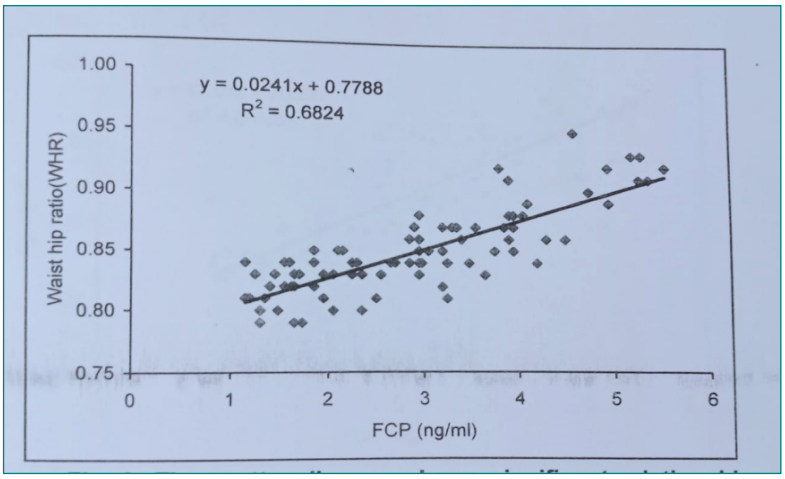

Figure 2. *The scatter diagram shows significant relationship (r=0.829) between FCP and waist hipratio (WHR).*

Fasting C-peptide (FCP) of 92 cases was expressed in ng/ml and Triglyceride (Tg) was expressed in mg/dl. Significant positive correlations were found between fasting C-peptide (FCP) and triglyceride (Tg). The values of Pearson's correlation coefficient were 0.862 in fasting C-peptide (FCP). This was significant ($p<0.05$). Therefore, there was linear positive correlation between fasting C-peptide (FCP) and triglyceride (Tg) in the study population (Figure 3).

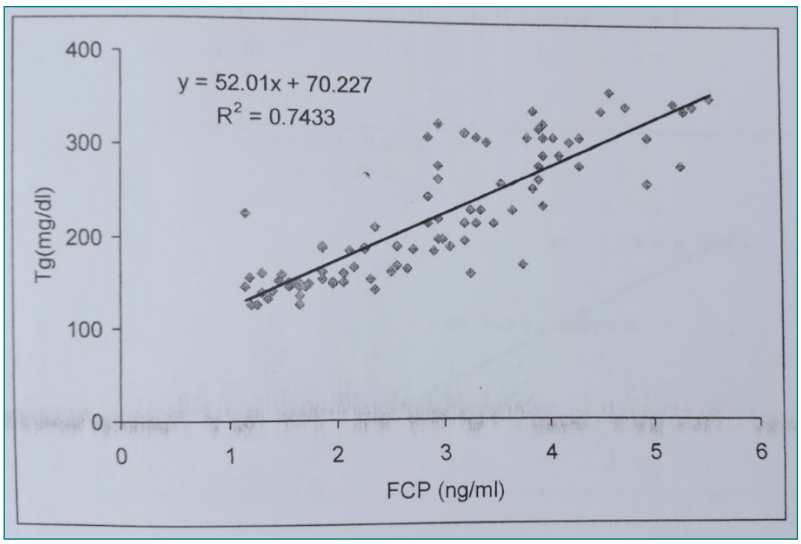

Figure 3. *The scatter diagram shows significant relationship (r=0.862) between FCP andtriglyceride (Tg).*

Fasting C-peptide (FCP) of 92 cases was expressed in ng/ml and grade was measure. Significant positive correlations were found between fasting C-peptide (FCP) and grade. The values of Pearson's correlation coefficient were 0.873 in fasting C-peptide (FCP). This was significant ($p<0.05$). Therefore, there was linear positive correlation between fasting C-peptide (FCP) and grade in the study population (Figure 4).

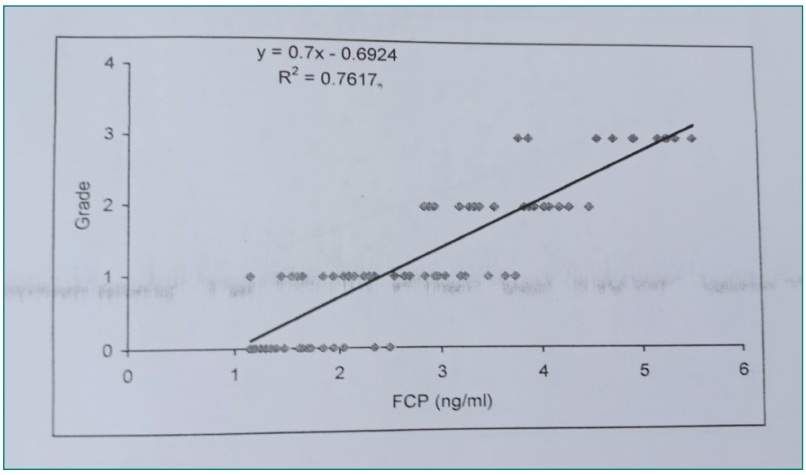

Figure 4. *The scatter diagram shows significant relationship (r=0.873) between FCP and grade(Liver echogenicity).*

Fasting C-peptide (FCP) of 92 cases was expressed in ng/ml and body mass index (BMI) was expressed in kg/m². Significant positive correlations were found between fasting C-peptide (FCP) and body mass index (BMI). The values of Pearson's correlation coefficient were 0.632 in fasting C-peptide (FCP). This was significant ($p<0.05$). Therefore, there was linear positive correlation between fasting C-peptide (FCP) and body mass index (BMI) in the study population (Figure 5).

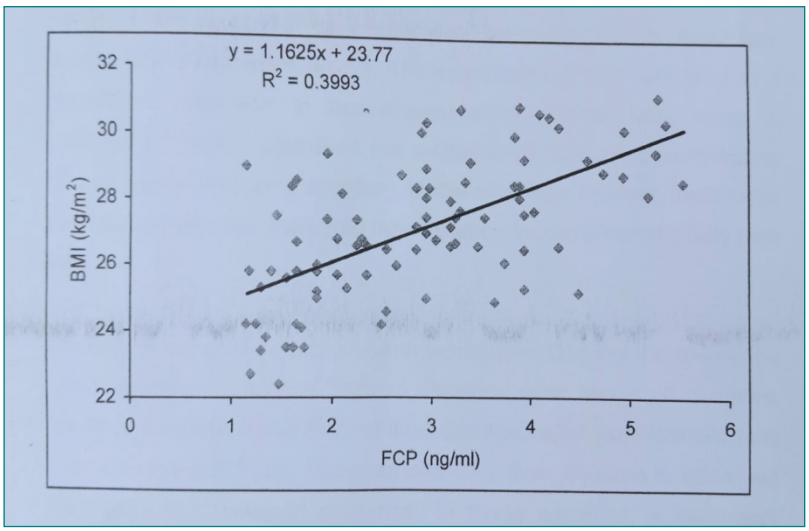

Figure 5. *The scatter diagram shows significant relationship (r=0.632) between FCP and BMI.*

12. Discussion

Ultrasonography is the cheapest, easily available, non-invasive tool that remains the modality of choice for the detection of fatty infiltration in liver. Though newer imaging modalities like computed tomography (CT) and magnetic resonance imaging (MRI) are used to evaluate hepatic steatosis, but these are costly and are not easily available. On the other hand, liver biopsy is the gold standard for the diagnosis of fatty liver, but being an invasive procedure, most of the clinicians avoid biopsy. So, ultrasonography is the most popular diagnostic modality for the diagnosis of liver diseases. Metformin increases insulin-mediated glucose utilization in peripheral tissues and has an anti-lipolytic effect that lowers serum free fatty acid concentrations. Metformin is found to cause a decrease in serum insulin and c-peptide concentrations by producing a significant improvement in insulin action. The improvement probably lead to a significant reduction in the accumulation of free fatty acids in hepatocytes and suppressed the oxidation of fatty acids contributing to cell injury and inflammation. Metformin may prevent, restrict or reverse hepatic steatosis and inflammation in non-alcoholic fatty liver disease. Obesity is a major health problem worldwide. Obesity increases the Obesity is a risk of developing several chronic diseases such as type II diabetes, insulin resistance and a form of liver disease called nonalcoholic fatty liver disease (NAFLD). Nonalcoholic fatty liver disease is observed principally in developed countries. In these societies, a sedentary lifestyle and high calorie, sugar, and fat intake lead to a high prevalence of obesity, insulin resistance, and diabetes. The risk for non-alcoholic fatty liver disease increases with increased obesity. Therefore patients with morbid or malignant obesity are at even higher risk from this disease. The relation between fatty liver and impaired glucose tolerance, diabetes mellitus and hyperlipidaemia has been well established. Overweight, hyperlipidaemia, and diabetes mellitus are high risk factors for fatty liver. It is clear that NAFLD is a chronic liver disease with the potential for progression to cirrhosis and to cause liver- related death. This cross sectional study was carried out at BIRDEM from May, 2006 to April, 2007 with the aim to investigate the correlation between gradings of hepatic echogenicity (grade-0, grade-1, grade-2 and grade-3) and serum c-peptide and triglyceride level in patients suffering from type2 diabetes treated with metformin. Another aim was to observe the association of serum c-peptide level to serum Trigleceride level, body mass index (BMI), waist hip circumference (WHR) and to the severity of liver echogenicity. The study was conducted in 92 patients, selected from both sexes between 44 and 68 years of age. In this study, the mean age of patients in grade-0 was 52.3 ± 1.0 years with age range 44-60 years, mean age of grade-1 was 55.7 ± 1.0 years with age range 46- 68 years, mean age of grade-2 was 58.5 ± 1.1 years with age range 48-68 years and mean

age of grade- 3 was 58.3 ± 1.4 years with age range 50-65 years. There appears a significant (P< 0.05) age difference among four groups. The meanage in different groups was almost similar in the previous study conducted by Papanas et al [9]. In the present study, the mean fasting c-peptide level in grade-0 patients was 1.59 ± 0.09 ng/ml in grade-1 was 2.40 ± 0.12 ng/ml, in grade-2 was 3.59 ± 0.10 ng/ml and in grade-3 was 4.88 ± 0.18 ng/ml. The mean of fasting c-peptide showed significant difference between patients of grade-0, grade-1, grade-2 and grade-3 liver echogenicity and similar positive correlation was also found in the previous study conducted by Papanas et al [9]. The mean serum triglyceride (Tg) level was 144.90 ± 2.67 mg/dl in grade-0, 181.60 ± 4.80 mg/dl in grade-1, 279.90 ± 8.23 mg/dl in grade-2 and 321.2 ± 10.55 mg/dl in grade-3 groups. The mean of triglyceride (Tg) showed a significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity, which was also similar as that found by Papanas et al [9] and Uygun et. Al [10] in previous studies. Mean waist hip ratio (WHR) in grade-0 was 0.81 ± 0.003, in grade-1 was 0.84 ± 0.002, in grade-2 was 0.86 ± 0.002 and in grade-3 was 0.92 ± 0.005. The mean of waist hip ratio showed a significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity and revealed the similar results as found by Papanas et al [9]. The mean body mass index (BMI) in grade-0 was 24.73 ± 0.35 kg/m², in grade-1 was 26.67 ± 0.24 kg/m², in grade-2 was 28.29 ± 0.30 kg/m² and in grade-3 was 29.85 ± 0.38 kg/m². The mean body mass index showed a significant difference between grade-0, grade-1, grade-2 and grade-3 liver echogenicity. The result of previous study conducted by Uygun et al [10] and Papanas et al [9] found to be similar as that found in the present study. In multiple regression analysis, it was observed that waist hip ratio (WHR), serum triglyceride (Tg) levels, gradings of liver echogenicity and body mass index (BMI)- all had positively associated with higher fasting C-peptide (FCP) levels and similar result was also found by Papanas et al [9]. In this study, it was observed that excessive body weight (overweight and obesity) plays an important role in the development of fatty liver disease. It is seen that the more the BMI the more the fat infiltration in liver. In this study, it was observed that an increase in the waist hip ratio (WHR) increases the severity of fatty infiltration in liver. It was seen that the more the WHR, the more severe form of fatty change in liver. High WHR suggests increased abdominal fatty tissue, which is a strong predictive factor for diabetes mellitus and other metabolic abnormalities. Increased WHR is considered as the most important risk factor for fatty liver.

13. Limitation of the Study

The study was conducted among 92 subjects which is a small sample size and no follow-up was done. So, exact effects of metformin on non-alcoholic fatty liver disease could not be evaluated. So, further study is recommended using a larger sample size with proper follow-up.

14. Conclusion

This study concludes that severity of liver echogenicity correlates to c-peptide and triglyceride levels in type 2 diabetic patients who were being treated with metformin. Metformin is used to reduce blood glucose level in insulin resistant obese or overweight type2 diabetic patient. Metformin can also be used to reduce fatty infiltration in liver in obese type2 diabetic patient as well as obese non-diabetic patient. But no statistical data is available regarding its long-term effect on hepatic steatosis, which should be evaluated in larger controlled trials with extended follow-up in type2 diabetic as well as in non-diabetic patients suffering from hepatic steatosis.

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