ORIGINAL ARTICLE

A Study of Insulin Resistance and its Clinico-Metabolic Correlates by Modified Harano's Method in Euglycemic Cirrhotics

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Abstract

To study insulin resistance and its clinico metabolic correlates in 20 euglycemic cirrhotics by Modified Harano's method. 20 euglycemic cirrhotics (18 male and 2 female; 14 alcoholic & 6 post hepatitis B) and equal number of age, sex and BMI matched healthy controls of mean ± SD age 37.1±8.35 v/s 36.5±7.48 years and BMI 18.4±1.83 v/s 18.5±1.77 kg/m2 were enrolled. All subjects underwent 75 gram oral glucose tolerance test (OGTT) in a fasting state. Samples for glucose (0,30,60, 90 & 120 min) and insulin (0 & 120 min) were withdrawn. Assessment of insulin resistance was carried out by Insulin suppression test (Modified Harano's method) i.e. simultaneous infusion of glucose as 20% dextrose @ 6 mg/kg/min and plain human insulin @ 50 mU/kg/hr. Steady state plasma glucose (SSPG) and steady state plasma insulin (SSSI) of 120-150 min of infusion were determined. Metabolic clearance rate for glucose (MCR) calculated as rate of glucose infusion/SSPG and insulin clearance rate (ICR) as rate of insulin infusion/SSSI. Lower the MCR and ICR, higher is the state of IR. Correlations of MCR and ICR with various clinical and metabolic variables were then studied. The result of present study suggests that cirrhosis is an insulin resistant state with low clearance rates for glucose and insulin, significant higher levels of glucose and insulin in postprandial state and markedly low levels of lipids. The state of insulin resistance is independent of severity of hepatic decompensation as classified by Child-Pugh's classification, etiology, nutritional status, clinical state, liver function tests and serum lipids.

Key words

Insulin resistance, cirrhosis, Modified Harano's method, metabolic clearance rate

Introduction

Liver has a key role in glucose metabolism. It maintains normal levels of blood glucose by a combination of glycogenesis, glycolysis, glycogenolysis and gluconeogenesis. These pathways are regulated by a number of hormones including insulin, glucagon, growth hormone and catecholamines (1). Also liver is the major organ metabolizing insulin. Disturbed homeostasis of glucose and insulin occur more frequently in liver cirrhosis than in general population. More than half of the patients with cirrhosis show abnormal glucose tolerance (2) and about one third overt diabetes (2,3,4)].

Insulin resistance (IR) is an important pathophysiological phenomenon causing impaired glucose tolerance (IGT) and diabetes mellitus (DM). Following mechanism explain insulin resistance in cirrhosis (5) : Insulin receptor/post receptor dysfunction, hyperinsulinemia, down regulation of insulin receptors, elevated insulin counteracting hormones, cytokine concentration, and lack of insulin like growth factors.

A lot of work to investigate insulin resistance in impaired glucose tolerant and diabetic cirrhotics has been done. However only few studies are available documenting insulin resistance in normal glucose tolerant and non diabetic cirrhotics particularly in our country (4). We decided to study in 20 euglycemic cirrhotics : anthropometric and metabolic profile, and association, if any with IR, glucose and insulin responses to oral glucose load, insulin resistance, and correlation of insulin resistance with severity of cirrhosis as classified by Child-Pugh's classification, etiology of cirrhosis, nutritional status, clinical state, biochemical parameters of liver function and serum lipids.

Materials and Methods

We recruited 20 stable euglycemic cirrhotic patients. The diagnosis of cirrhosis was based on histopathological evidence (liver biopsy) or unequivocal clinical grounds (chronic liver disease stigmata, jaundice, ascites, esophageal

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varices), impaired liver function tests and ultrasonographic features consistent with cirrhosis (diffuse alteration and nodular transformation of liver parenchyma, and signs of portal hypertension). 20 age (± 2 yrs), sex and BMI (± 2 kg/m2) matched healthy subjects were recruited as controls. Controls were healthy relatives of non diabetic patients admitted in the hospital. Subjects with diabetes, impaired glucose tolerance, family history of diabetes, co-existing illnesses, current alcoholics and smokers were excluded. Drugs like diuretics and b-blockers if being used were withdrawn at least a week before taking up for study. A thorough history taking, clinical examination, and biochemical investigation were followed by ultrasonography and endoscopy. Thereafter, all subjects underwent standard oral glucose tolerance test (OGTT) after an overnight fast. Blood samples were drawn for glucose and insulin every 30 minutes for 2 hours. After a gap of 2-3 days, all subjects underwent insulin suppression test by Modified Harano's method (6). After an overnight fast, insulin (regular human Actrapid, Novo Nordisk) @ 50 mU/kg/hr and glucose as 20% dextrose, @ 6 mg/kg/min were infused through an I/V catheter placed on right arm by a syringe infusion pump (Model Pilot A2, Fresenius, Germany) and a volumetric infusion pump (cure mate Model SM 2100, Jong Sang Techno Co. Ltd, Korea) respectively. The infusion was continued till 150 min. After achievement of steady state at 120 min, samples for glucose and insulin were withdrawn serially at 5 min interval (total of seven samples) from the other arm. The mean concentration of plasma glucose and serum insulin in the seven samples (120, 125, 130, 135, 140, 145, 150 minutes) were calculated representing steady state plasma glucose (SSPG) and steady state serum insulin (SSSI). Metabolic clearance rate for glucose (MCR) and insulin clearance rate (ICR) were calculated as rate of glucose infusion/SSPG and rate of insulin infusion/SSSI respectively. Low MCR and ICR, and high SSPG and SSSI suggest a state of higher IR. Correlations of MCR with various clinical and metabolic parameters were studied.

Study was conducted at the department of medicine and biochemistry, Lady Hardinge Medical College and Dr. Ram Manohar Lohia Hospital, New Delhi. Insulin levels were measured by radioimmunoassay (Coat-A-Count Insulin kits of Diagnostic Products Corporation, US) at RIA Lab, Institute of Nuclear Medicine and Allied Sciences, Delhi. Total cholesterol (TC), high density lipoproteins (HDL) and triglycerides (TG) were measured directly. Very low density lipoproteins (VLDL) and LDL were calculated indirectly as TG/5 and TC - (HDL + VLDL) respectively. Written informed consent of each subject was taken. Study was approved by the ethical committee of the institution.

All data are expressed as means ± SD, and analysis was performed using the SPSS 10.0 package for windows. A p value of less than 0.05 was considered statistically significant.

Results

Physical and Biochemical characteristics : (Table 1&2)

Serum lipids : Table 3 compares serum lipid levels of cirrhotics and healthy controls. All serum lipids i.e.,TC, VLDL, LDL, TG and HDL were significantly lower in cirrhotics (all $p < 0.01$). For TC, LDL and HDL, no two Child's classes were different at 5% statistical significance level, but mean VLDL and TG of Child's A were statistically significantly higher as compared to combined Child's B and C ($p < 0.05$) (VLDL - Child's A: 16.5 ± 2.1; B: 10.6 ± 2.4; C: 10.4 ± 2.1 mg/dl and TG - Child's A: 83.0 ± 10, B: 53.0 ± 13; C: 51.2 ± 11 mg/dl). The negative correlation between TG and VLDL with Child's score narrowly missed statistical significance at 5% level (for TG: $r = -0.42$ & VLDL: $r = -0.42$, ($p < 0.05$ for $r = 0.44$)). No significant correlations were achieved between various liver function tests (LFT) and serum lipids except for significant negative correlation between prolonged prothrombin time (PT) (seconds deranged) and TG & VLDL (for TG: r -0.50 & VLDL: $r = -0.48$, $p < 0.05$).

Oral glucose tolerance test parameters : The fasting, 30 & 60 min plasma glucose of cirrhotics were not statistically different from healthy controls ($p > 0.05$). But 90 and 120 min plasma glucose of cirrhotics were significantly higher as compared to controls (90 min: 134.1±26.1 v/s 117.0± 25.5 mg/dl & 120 min: 111.7±23.8 v/s 94.8±26.7 mg/dl, $p < 0.05$) (Figure 1). The fasting insulin of cirrhotics and controls was comparable (12.5±8.5 v/s 8.5±7.2 mIU/ml, $p > 0.05$) but 120 min insulin in cirrhotics was significantly higher (34.5 ±16.3 v/s 20.0±14.2 mIU/ml, $p < 0.05$). The fasting and 120 min glucose/insulin (GI) ratios of cirrhotics were significantly lower as compared to healthy controls (100.2±51.9 v/s 148.4±74.5 mg/mu and 39.3±21.4 v/s 61.5±34.6 mg/mu respectively, $p < 0.05$).

Insulin suppression test (Modified Harano's method) parameters : The steady state plasma glucose (SSPG) and steady state serum insulin (SSSI) of cirrhotics were significantly higher when compared to controls (all $p < 0.001$) (Figure 2). The cirrhotics had markedly low

clearance for glucose as well as insulin during the study (low MCR and ICR, all p £ 0.001). These results are shown in Table 4. The MCR and ICR of alcoholic (n=14) and post necrotic (hepatitis B) (n=6) cirrhotics were not found to be statistically different (MCR: 4.94±1.6 v/s 4.99±3.1 and ICR: 10.3±10.1 v/s 11.7±3.5 ml/kg/min respectively (p >0.05)). S.albumin was not found to be significantly correlated with either MCR (r= -0.04, p >0.05) or ICR (r= - 0.03, p >0.05). Also mid arm circumference was not significantly correlated with MCR (r= 0.23, p > 0.05) or ICR (r= - 0.07, p > 0.05). The MCR of cirrhotics with no ascites, mild, moderate and severe ascites was not found to be different statistically (p > 0.05). The MCR was also not found to be significantly correlated with severity of ascites (r=0.33, p >0.05). ICR was found to be negatively correlated with ascites in cirrhotics, the correlation being statistically significant (r= -0.55, p <0.01). The mean ICR of subjects with no ascites was 22.02±18.0 ml/kg/min, with mild ascites was 10.63±6.6 ml/kg/min, with moderate ascites was 11.03±3.4 ml/kg/min and with severe ascites was 6.90±2.6 ml/kg/min. ICR of cirrhotics with no ascites was significantly higher than cirrhotics with severe ascites (p <0.001). The mean ICR of Child's class A was 13.88±8.4 ml/kg/min, of Child's class B was 13.65±13.2 ml/kg/min, and of Child's class C was 8.29±3.6 ml/kg/min. No two groups were different at 0.05 significance level. ICR achieved negative correlation with Child's score that narrowly missed statistical significance at 0.05 level (r= -0.41, p < 0.05 for r ³ 0.44). The MCR and ICR of cirrhotics with different grades of oesophageal varices were compared by one way analysis of variance (within groups) followed by student t test (between groups). No two groups were found to had any statistical difference at 0.05 significance level (MCR: No varices: 4.72±1.4; grade I: 5.39±3.5; grade II: 4.80±1.6; grade III 5.46±4.2, and ICR: No varices: 4.73±1.4; grade I: 5.39±3.6; grade II: 4.81±2.6; grade III: 5.46±4.3 ml/kg/min). S. bilirubin, ALT, AST, ALP and PT (seconds prolonged) were not statistically significantly correlated with either MCR or ICR (p >0.05). Various serum lipids (TC, VLDL, LDL, TG and HDL) were not found to had any statistically significant correlation with MCR or ICR (p >0.05).

The mean MCR and ICR of cirrhotics with different Child's class i.e. A (n=2), B (n=7) and C (n=11) were compared. The MCR was 4.87 ±2.9, 5.40±1.9 and 4.86±2.3 ml/kg/min for Child's class A, B and C respectively. No two groups were found to be different at 0.05 level. The MCR of cirrhotics with combined Child's class A+B (n=9, Child's score £ 9) was not different statistically from those with Child's class C (n=11, Child's score >9) (5.08±1.9 vs 4.86±2.3 ml/kg/min respectively, p > 0.05). The ICR of Child's class A was 13.88±8.4, of B was 13.65±13.2, of C was 8.29±3.6 ml/kg/.min. No two groups were different at 0.05 level. Also, MCR (r= -0.09) and ICR (r= -0.41) were not found to be significantly correlated with Child's score (p >0.05).

Table 1. Physical characteristics of cirrhotics and healthy controls

Physical characteristic	Cirrhotics	Controls	p value
Number	20	20	
Mean±SD age (years)	37.15±8.35	36.50±7.48	> 0.05
Male/Female	18/2	18/2	
Body mass index (kg/m2)	18.36±1.83	18.49±1.77	> 0.05
Waist hip ratio	0.99±0.10	0.84±0.06	<0.001
Mid arm circumference (cm)	21.05±2.85	24.62±2.07	<0.001
Systolic B.P (mmHg)	111.95±18.16	121.50±10.26	<0.05
Diastolic B.P (mmHg)	69.10±10.10	78.40±5.17	<0.001

Table 2. Haemogram and metabolic profile

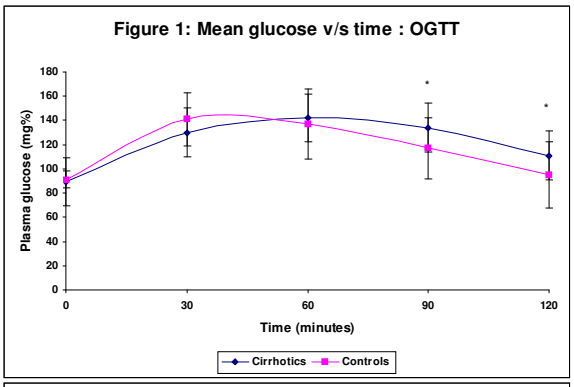
Parameters (Mean±S.D)	Cirrhotics	Controls	p value
Haemoglobin (gm%)	9.25±2.28	13.73±1.160	<0.05
Platelet count (cells/mm^3)	168.20 ± 83.63x10^3	249.95 ± 55.66x10^3	<0.05
S. bilirubin (mg%)	2.47±2.27	0.49±0.29	<0.05
Prothrombin time (sec)	8.94±5.76	0.98±0.95	<0.05
(ALT) IU/l	48.83±25.33	26.20±12.27	<0.05
Aspartate	66.45±48.59	28.60±11.36	<0.05
(ALP) IU/l	160.32±51.54	100.63±36.26	<0.05
S. albumin (gm%)	3.44±0.51	3.28±0.44	>0.05
Albumin/globulin ratio	1.32±0.35	1.24±0.22	>0.05
S. urea (mg%)	24.60±11.70	24.20±7.37	>0.05
S.creatinine (mg%)	0.86±0.25	0.80±0.15	>0.05
Sodium (meq/l)	134.80±7.34	141.65±5.97	<0.05
Potassium (meq/l)	3.94±0.48	4.38±0.56	<0.05

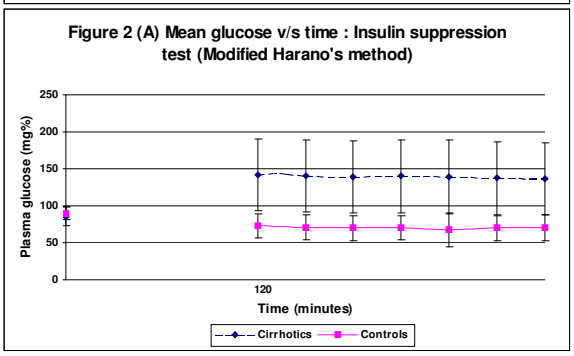
Table 3. Serum lipid profile (Mean±S.D)

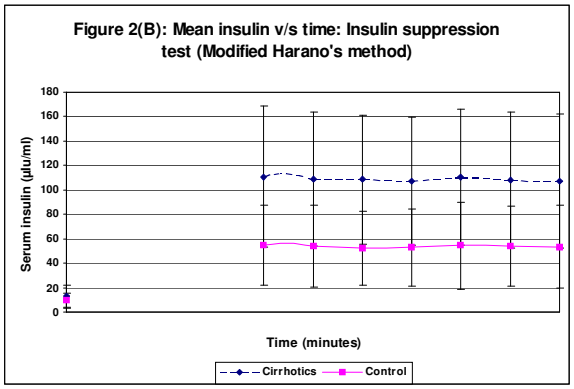
Serum lipid (mg%)	Cirrhotics	Controls	p value
TC	95.70±33.97	143.30±30.12	<0.001
VLDL	11.05±2.80	16.15±6.68	<0.01
LDL	62.95±28.84	92.35±23.45	<0.001
TG	55.0±14.71	80.75±33.68	<0.01
HDL	21.70±9.20	35.25±10.44	<0.001

Table 4. Insulin suppression test parameters (Mean±S.D)

Parameter	Cirrhotics	Controls	p value
Fasting glucose (m%)	85.6±12.6	89.8±8.7	>0.05
Fasting insulin (mIU/ml)	13.30±8.8	9.66±6.2	>0.05
SSPG (mg%)	139.25±49.15	70.66±16.67	<0.001
SSSI (mIU/ml)	108.72±54.5	53.71±32.3	<0.001
MCR (ml/kg/min)	4.96±2.13	8.90±1.87	<0.001
ICR (ml/kg/min)	10.73±8.5	22.87±13.1	= 0.001







Discussion

As cirrhotics and controls were matched for BMI, there was no significant statistical difference. However true BMI in cirrhotics may not be estimated accurately due to presence of ascites and oedema in majority (17 out of 20) of our patients. Mean waist hip ratio (WHR) of cirrhotics was significantly higher as compared to controls. As most of the cases were decompensated and had ascites (17 / 20), WHR is likely to be inflated. Anthropometry and even bioelectrical impedence do not accurately reflect changes in body composition associated with cirrhosis particularly in the presence of ascites (7). Such situation requires the use of direct methods such as in vivo neutron activation analysis, dual energy X-ray absorptiometry or deuterium oxide dilution (7).

The cirrhotics had significantly lower mid arm circumference (MAC). As we know that MAC gives an estimate of lean body mass or muscle protein and is a better marker for nutrition in cirrhotics as compared to BMI or WHR. McCullough *et al* reported that in cirrhotics, protein catabolism was increased in the baseline post absorptive state and it failed to suppress normally in response to infusion of amino acids, either alone or in combination with insulin. This suggests an insulin resistance for protein metabolism (8).

The blood pressure both systolic and diastolic, were significantly lower in cirrhotics. suggesting that cirrhosis is a hypotensive state. This impairment may be explained by the intracellular actions of vasorelaxant substances which are over produced in cirrhotics (9). The patients of cirrhosis had significantly lower haemoglobin, decreased platelet count, raised serum bilirubin, prolonged prothrombin time, raised liver enzymes (ALT, AST and ALP) but comparable serum urea and creatinine levels. These findings are consistent with cirrhosis as a disease entity. However serum albumin in cirrhotics (3.44±0.5 v/s 3.28±0.4 gm%) was not different from that of controls. Leatherdale BA et al also reported comparable s.albumin of cirrhotics and healthy controls (10).

All measured serum lipids (TC, VLDL, LDL, HDL and TG) were significantly lower in cirrhotics. We suggest that cirrhosis is a state of hypolipidemia. This may be due to the fact that cirrhosis is a diffuse parenchymal disease causing poor hepatic synthesis of lipids and lipoproteins (11). Also, cirrhosis is a hypermetabolic state with increased energy needs resulting in increased utilization of lipid stores (5). Owen *et al* reported that in cirrhotics, higher rate of calories were derived from fat i.e. increased lipolysis (69% in cirrhotics v/s 40% in controls) and at the same time a lower rate of calories were derived from carbohydrates (13% in cirrhotics, 39% controls) (12). Furthermore cirrhotics may have adipose tissue and lipid metabolism insensitivity to insulin (13). An interesting finding was noted in our study that serum triglycerides levels were significantly higher in Child's class A cirrhotics when compared with combined Child's class B and C. This may be due to preserved ability of liver to synthesis VLDL in Child's A cirrhotics as compared to child's class B & C.

As stated earlier, we included only normal glucose tolerant cirrhotics in our study. Cirrhotics had significantly higher 90 min and 120 min (post 75 gram glucose load) plasma glucose, and 120 min serum insulin. This suggest that even euglycemic cirrhotics have

significantly higher postprandial glucose and insulin response to oral glucose challenge. It appears that more than normal amount of insulin is required to produce glucose homeostasis. Thus a state of decreased insulin sensitivity or endogenous insulin resistance is present in cirrhosis which probably precedes appearance of IGT and frank diabetes. Jain carried out OGTT in normal glucose tolerant cirrhotics (4). They reported comparable fasting glucose, postprandial glucose and fasting insulin but significantly higher postprandial insulin. Kruszynska *et al* also reported higher postprandial glucose, fasting insulin and postprandial insulin in cirrhotics with fasting glucose being normal (14). Hyperinsulinemia in cirrhotics may be because of decreased hepatic extraction (15), increased b cell response leading to hypersecretion of insulin (15), and portal hypertension leading to portocaval shunting and bypassing of liver (16).

Both fasting and postprandial (120 min) glucose/insulin (GI) ratio in cirrhotics were significantly lower when compared with controls. Thus cirrhotics had higher concentration of insulin for similar levels of plasma glucose, thus implying a state of insulin resistance. Fulcher *et al* suggested that a direct comparison of GI ratio can provide a rough guide to insulin action (17). The greater the insulin response to glucose, the more insulin resistant the subject is if glucose level remain the same (17). Also Komshian *et al* demonstrated excellent correlation between fasting GI ratio with insulin resistance derived from Insulin suppression test and Euglycemic clamp technique (18).

MCR has been widely used as a measure of insulin sensitivity and it remain constant over a wide range of plasma glucose concentration. It is independent of the level of glycemia at which subject are being compared (17). Lower the MCR, lower is the glucose disposal or utilization and reduced response to insulin, thus implying insulin resistance. In our study, MCR was found to be significantly lower in cirrhotics as compared to healthy controls (4.96±2.1 v/s 8.90±1.9 ml/kg/min. Thus it may concluded that euglycemic cirrhotics have significant degree of IR.

Iversion *et al* reported significantly reduced net glucose metabolism in cirrhotics as compared to controls (53±9 v/s 72±16 mmol/kg/min. They concluded that IR in cirrhosis is caused both by a marked decrease in sensitivity to insulin and decreased maximum effect of insulin indicating a combined receptor postreceptor defect as underlying cause (19). Shmueli *et al* also reported significantly lower total glucose utilization in cirrhotic subjects (3.9±0.3 vs 8.8±1.7 mg/kg/min. They suggested that this deficiency was accounted for by lower non oxidative glucose disposal (20). Selberg *et al*, with the help of euglycemic clamp technique, indirect calorimetry and positron emission tomography (PET) scan analysis of skeletal muscle glucose metabolism, studied insulin resistance in cirrhotics. They found that both whole body and non-oxidative glucose disposal were significantly reduced in patients with cirrhosis (by 48%, and 79%. They concluded that cirrhosis is a insulin resistant state characterised by both decreased glucose transport and decreased non-oxidative glucose metabolism in skeletal muscle (21). Our study was designed only to estimate IR in euglycemic cirrhotics. The causes or site of IR in cirrhosis cannot be commented upon by this study.

Insulin clearance rate (ICR) was significantly low in cirrhotics when compared to controls (10.73±8.5 v/s 22.87±13.1 ml/kg/min. This suggests that cirrhotics had significant hyperinsulinemia and low clearance for insulin, thus implying that cirrhosis is a state associated with enhanced IR. Kruszynska *et al* reported 34% decrease in ICR (8.3±0.6 v/s 12.5±0.5 ml/kg/min (15). They suggested b cell hypersecretion and reduced hepatic insulin clearance as possible causes. Yoshida and coworkers performed percutaneous transhepatic portal vein catheterization, and measured splanchnic output and portal vein insulin concentration (22). They demonstrated 50% reduced metabolic clearance rate for insulin resulting from decreased insulin clearance by the liver. On the contrary, Magnusson *et al* reported unchanged insulin clearance in cirrhosis (18.0±2.0 v/s 17.0±2.5 ml/kg/min in controls (23).

According to presently available data, portosystemic shunting does not primarily contribute to the reduced degradation of insulin. It is the parenchymal damage which leads to decreased hepatic metabolisation of insulin thus resulting in hyperinsulinemic state (24,25).

The MCR and ICR of different Child's grade A, B and C were not found to be statistically different at 0.05 level. Thus it may be concluded that severity of hepatic decompensation as assessed by Child-Pugh's classification is not related to insulin resistance in cirrhosis. We also observed that etiology of cirrhosis i.e, alcoholic (n=14) v/s postnecrotic (n=6) does not seem to affect IR. MCR and ICR of both alcoholic and post necrotic cirrhotics were not different statistically.

Nutritional parameters like serum albumin and mid arm circumference were not found to be significantly related to parameters of IR. On analyzing correlations between MCR and degree of ascites results were not significant. However ICR was significantly negatively correlated with degree of ascites though negative correlation with Child's score narrowly missed statistical significance at 0.05 level (r= -

0.41, p <0.05 for r ³ 0.44). We could not demonstrate any significant relationship between different grades of varices and IR. Also various liver function tests like bilirubin, ALT, AST, ALP and deranged PT were not significantly related to IR. Similarly various serum lipids measured were not found to had any statistically significant correlation with parameters of IR. These observations suggest that IR in cirrhosis is independent of etiology, nutritional status, clinical state, biochemical parameters of liver function and serum lipids. However low clearance of insulin (low ICR) suggestive of a state of insulin resistance was significantly associated with severity of ascites. Also Child's class of the cirrhotics does not affect IR. Muller *et al* also reported that IR in cirrhosis is independent of liver function, clinical and nutritional status of the patients with cirrhosis (26).

In conclusion, we suggest that cirrhosis is an insulin resistant state which may be responsible for increased incidence of impaired glucose tolerance and hepatogenous diabetes. The state of IR in cirrhosis is not affected by the Child's class, etiology of cirrhosis, nutritional status, clinical state, liver function tests and serum lipids.

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