Effect of interferon therapy on glucose metabolism in children with chronic hepatitis B

Zarife Kuloğlu[1], Merih Berberoğlu[2], Aydan Kansu[1], Fulya Demirçeken[3], Tümay Doğancı[4], Gönül Öcal[2], Nurten Girgin[1]

Departments of [1]Pediatric Gastroenterology and [2]Pediatric Endocrinology, Ankara University Faculty of Medicine, and [3]Dr. Sami Ulus Children's Hospital, and [4]Dışkapı Research and Training Hospital, Ankara, Turkey

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The aim of this study was to investigate the effect of interferon (IFN)-α treatment on glucose metabolism in children with chronic hepatitis B (CHB). Forty children with CHB received IFN 10 MU/m² for six months. Oral glucose tolerance test, anti-insulin and anti-glutamic acid decarboxylase (GAD) antibody, fasting plasma C-peptide and insulin (FPI), postprandial insulin, homeostasis model assessment of insulin resistance (HOMA–IR), HOMA-cell, and glucose/insulin ratio (G/I) were measured before and after treatment. The last four parameters were also evaluated in healthy controls (n=42). In patients, fasting plasma glucose (FPG) and HOMA-IR levels were significantly lower than in controls (p=0.001 and p=0.020, respectively). There was a strong correlation between degree of liver disease and FPG. Two patients had hyperinsulinemia. HOMA-IR was suppressed in 7 patients enough to indicate increased sensitivity. FPI of 13 patients and HOMA-cell of 9 patients were lower than the minimum level of controls, features compatible with β-cell hypofunction. Frequency of glucose metabolism abnormalities was not different before and after therapy. After therapy, only 1 patient developed anti-GAD antibody, and FPI of 8 children and HOMA-cell level of 9 children were lower than the minimum level of controls. Hyperinsulinemia was persistent in the same patients. We demonstrated that HBV-infected children had insulin sensitivity; however, no adverse effects of IFN on glucose homeostasis were seen.

Key words: interferon, chronic hepatitis B, children, glucose metabolism.

The liver is one of the principle organs involved in glucose metabolism. Previous studies have shown that chronic viral infections such as human immunodeficiency virus and hepatitis C virus (HCV) have a link with the derangement of glucose homeostasis and the induction of insulin resistance (IR)[1-4]. However, the relationship between chronic hepatitis B (CHB) virus infection and glucose metabolism remains unclear.

Interferon-α (IFN-α) has been widely used in the treatment of children with CHB[5]. In recent years, however, attention has been drawn to the possible effects of IFN-α on glucose homeostasis[7-9]. On the one hand, IFN-α has been reported to induce type 1 diabetes mellitus (DM) and acute IR in patients with chronic HCV infection[6-10], while on the other hand, some studies claim that, in patients with HCV infection, rather than causing deterioration of insulin sensitivity and glucose tolerance, IFN-α treatment is capable of improving glucose tolerance[11-13]. Little information is available on this issue in CHB. Triggering type 1 DM development has been reported as an adverse effect of IFN-α treatment[14]. Although deterioration in diabetes control during IFN-α treatment for HBV has been reported, the beneficial effect of IFN-α on glucose homeostasis in patients with HBV infection has also been reported[15,16]. These studies involve adults, and the first information about the effects of IFN on glucose metabolism in CHB in childhood was reported

by us previously[17]. In that report, we presented preliminary results of this prospective trial in a group of 14 CHB patients treated with high-dose IFN-α therapy. We demonstrated that most of the children with CHB had hypoinsulinemia and insulin hypersensitivity; however, IFN-α had minimal positive impact on glucose metabolism. The current study is a continuation of this previous study. The aim of this study was to determine the prevalence of disturbance of glucose metabolism in children with CHB and to investigate the effect of IFN-α treatment in a larger group of patients.

Material and Methods

Patients and Study Design

Forty children with CHB and 42 healthy children were enrolled subsequently in the current report. Children with CHB received 10 megaunits/m^2 IFN-α, subcutaneously, three times per week for six months. Liver biopsy was performed in all patients before starting the therapy. Biopsy specimens were evaluated according to Knodell's histologic activity index (HAI) (18). Mild hepatitis was accepted as HAI score between 4 and 7, moderate hepatitis as HAI score between 8 and 12, and severe hepatitis as HAI score >12. The patients were all positive for hepatitis B surface antigen (HbsAg) and hepatitis B e antigen (HbeAg) in serum for at least six months, hepatitis B virus DNA (HBV DNA) level >5 pg/ml, serum alanine aminotransferase (ALT) level at least 1.5 times the upper limit of normal (ULN) range (37 U/L) but <500 IU/L, and HAI ≥4. None of the patients had clinical or biochemical evidence of decompensated liver disease, hepatitis delta, hepatitis C, or human immunodeficiency virus (HIV) coinfection, or contraindication for IFN therapy. Patients were also excluded if they had obesity, history of pancreatitis or DM and were taking medications known to affect glucose tolerance or insulin secretion.

Before and after IFN therapy, oral glucose tolerance test (OGTT), fasting and 120 minute (min) (post OGTT) insulin levels, fasting C-peptide levels, homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment-insulin secretion (HOMA-cell), fasting glucose/insulin ratio (G/I) anti-glutamic acid decarboxylase (anti-GAD) antibody, and anti-insulin antibody were evaluated. These tests were done within three days before and after completion of IFN therapy. Patients were followed monthly for complete blood count and biochemical, serological and virological parameters while on IFN-α therapy. Loss of HbeAg with hepatitis B e antibody (AntiHBe) seroconversion, normalization of ALT and clearance of serum HBV DNA were accepted as complete response.

Fasting plasma glucose (FPG), fasting plasma insulin (FPI) levels, HOMA-IR, HOMA-cell and G/I were also evaluated in age- and gender-matched healthy controls (n=42). The control group's body mass index (BMI) percentile was less than 80. None of them had family history of DM or were taking medications known to affect glucose tolerance or insulin secretion.

This study was approved by the local ethics committee of Ankara University, School of Medicine and was carried out in accordance with the Helsinki Declaration. Informed consent was obtained from the parents of all the patients before the study.

Assays of Glucose Metabolism

Oral glucose tolerance test (OGTT) was performed in the morning after a 12-hour (h) overnight fast using 1.75 g/kg oral glucose. Venous blood samples were taken just before the glucose load and after 30, 60, 90 and 120 minutes (min) for measurement of plasma glucose concentrations. During the OGTT, blood samples were also taken at 0 min for measurement of FPI and C-peptide concentrations and at 120 min for measurement of insulin concentration.

Oral glucose tolerance test (OGTT) results were evaluated according to the American Diabetes Association criteria[19]. Normal glucose tolerance was defined as FPG <100 mg/dl and 2-h plasma glucose concentration <140 mg/dl. Fasting glucose tolerance was defined as FPG between 100 mg/dl and 126 mg/dl, and 2-h plasma glucose concentration <140 mg/dl. Glucose intolerance was defined as FPG <126 mg/dl and 2-h plasma glucose concentration between 140 and 200 mg/dl. Diabetes was defined as FPG ≥126 mg/dl and 2-h plasma glucose concentration >200 mg/dl. Manifest hypoglycemia was defined as FPG

<60 mg/dl. Mild hypoglycemia was defined as FPG between 60 mg/dl and 70 mg/dl. Evaluation of the children's hyperinsulinemia included fasting and 120-min OGTT plasma insulin levels. FPI levels above 20 μIU/ml or 120-min (post-OGTT) insulin levels above 150 μIU/ml were accepted as hyperinsulinemia[20]. IR was evaluated through G/I and HOMA-IR. G/I ratio was calculated by FPG (mg/dl)/FPI (μIU/ml), which is expected to be above 6-7[21]. HOMA-IR was calculated using the following formula and the normal value is generally accepted as <3.5[22].

HOMA-IR = FPG (mmol/L) x FPI (μIU/ml) / 22.5

β-cell function was assessed by HOMA-cell, which reflects insulin secretion in basal condition. HOMA-cell was calculated using the following formula[23]:

HOMA-cell: [20xFPI (μIU/ml)] / [FPG (mmol/L) -3.5]

The BMI was calculated as weight (kg) divided by height (meter square). Obesity was defined as a BMI percentile >95[24].

Laboratory Analysis

Serum glucose was measured by using the glucose oxidase method. C-peptide level was studied with DSL-7000 kit (Diagnostic Systems Laboratories USA; reference values: 0.8-4 ng/ml), and serum insulin level was studied with Coat–A-Count kit (Diagnostic Products Corporation, Los Angeles, CA, USA; reference values: 3-15 μIU/ml) by radioimmunoassay (RIA) method[25,26]. Anti-GAD antibody was studied with glutamic acid Ab RIA/DA kit (CIS-Biointernational, USA; reference values: 0-1 U/L) by IRMA method, and anti-insulin antibodies were studied with anti-insulin Ab RIA/CT kit (Biosource, USA; reference values: 4-10%) by semiquantitative RIA method[27,28].

Statistical Analysis

All the values were calculated as mean ± standard deviation. The significance of intergroup differences was studied with SPSS 11.0 computer program by chi-square, Student's t, Wilcoxon signed rank and McNemar tests. $p<0.05$ was considered significant.

Results

Basic Characteristics

The mean baseline ALT, HBV DNA and HAI values of children with CHB were 116.6±79.1 U/L, 5107.5±7518.3 pg/ml and 7.9±2.8, respectively. ALT level in 16 patients was higher than 3 x ULN. Mild hepatitis was observed in 16 patients, moderate hepatitis in 21 and severe hepatitis in 3 patients.

In the control group, the mean FPI level was 10.7±4.1 μIU/ml (range 4.1- 19 μIU/ml), mean HOMA-IR level 2.3±1.1 (range 0.7-5), mean G/I level 9.1±3.5 (range 3.5-18.8), and mean HOMA-cell 182.7±97 (range 40-426). In children with CHB, FPG and HOMA-IR levels were significantly lower than in controls (p=0.001 and p=0.020, respectively). Patients with moderate/severe hepatitis on biopsy had significantly lower FPG level (71.9 ±16.5 mg/dl) than patients with mild hepatitis on biopsy (82.1±16.5 mg/dl, p=0.042). FPG

Table I. Demographic and Baseline Characteristics of Patients with Chronic Hepatitis B and Healthy Controls

	Patients with chronic hepatitis B	Healthy controls	p
Number	40	42	
Sex (Female/Male)	11/29	19/23	n.s.
Age (year)	9±4.1	10.4±2.7	n.s.
Body mass index	17.2±1.8	17.9±1.59	n.s.
Fasting plasma glucose (mg/dl)	76.1±17.1	87.8± 9.9	0.001
Fasting plasma insulin (μIU/ml)	8.9±6.2	10.7±4.1	n.s.
HOMA-IR	1.6±1.4	2.3±1.1	0.020
HOMA-cell	250.6±487.3	182.7±97.1	n.s.

Data are expressed as mean±SD
n.s.: Non-significant.

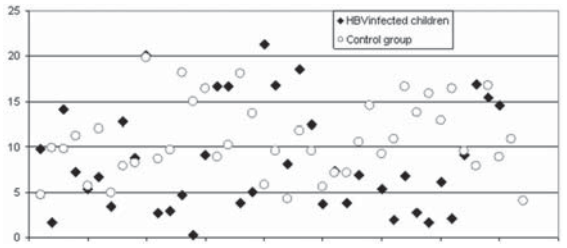

Fig. 1. Fasting plasma insulin levels in CHB-infected children and controls.

level was also significantly lower in those children who had elevated ALT >3 x ULN (67±18.2 mg/dl) than in those with ALT <3 x ULN (82±13.5 mg/dl) (p=0.005). Although G/I was higher in children with CHB than controls, it was not statistically significant. The baseline characteristics of patients are shown in Table I.

Efficacy of IFN-α Therapy

Initial mean ALT and HBV DNA values of patients significantly decreased to 56.2±32.1U/L and 1007.2 ± 1633.2 pg/ml at the end of therapy, respectively(p=0.001; p=0.02). At the end of therapy, ALT normalization and HBV DNA clearance were observed in 20 patients (50%), anti HBe seroconversion in 3 children (7.5%) and anti HBs seroconversion in 1 child (2.5%). Three of the 40 patients showed complete response to IFN-α at the end of therapy (complete response rate 7.5%). Twelve months after the end of therapy, ALT normalization was observed in 22 patients (55%), HBV DNA clearance in 20 patients (50%), anti HBe seroconversion in 14 patients (35%), and anti HBs seroconversion in 2 patients (5%). Complete response was achieved in 14 children (35%) 12 months after the end of IFN therapy.

Glucose Tolerance Before IFN Therapy

Oral glucose tolerance test (OGTT) was normal in 28 patients (70%). Hypoglycemia was present in 10 patients (25%). Seven of them had mild hypoglycemia. Fasting glucose intolerance was observed in 1 patient (2.5%) and glucose intolerance in 1 patient (2.5%). Children with hypoglycemia were significantly older than children without hypoglycemia (9.9±5.4 years vs 8.7±2.5 years, p=0.042). The patient with glucose intolerance had IR and hyperinsulinemia, and her family history was positive for type 2 DM. While 2 children had hyperinsulinemia (FPI >20 μIU/ml), FPI of 13 children (32.5%) was lower than minimum FPI level of the control group (Fig. 1). Fasting C-peptide levels were abnormal in 6 patients. Three of them had low fasting C-peptide level. High HOMA-IR levels (>3.5) were observed in 7 patients (17.5%). G/I was <6 in 9 patients (22.5%). HOMA-IR was suppressed in 13 children, and G/I was high in 9 children, enough to indicate increased sensitivity compared with minimum HOMA-IR and maximum G/I levels of the control group (Fig. 2). Nine children had lower HOMA-cell than the minimum level of the control group. Median HOMA-cell level was -75.5 (range (-1624.4) – (14.5)) in these children. None of the patients had anti-GAD or anti-insulin antibody positivity before treatment. Prevalence of glucose abnormalities are shown in Table II.

Glucose Tolerance After IFN Therapy

Posttreatment serum glucose levels of 30th, 60th and 120th min were significantly lower than those of pretreatment values (p=0.001, p=0.001 and p=0.001, respectively). No differences in the other parameters (FPG, fasting insulin and C-peptide, 120-min insulin, HOMA-cell, HOMA-IR and G/I) were detected between before and after IFN therapy values (Table III).

After IFN therapy, prevalence of glucose abnormalities decreased from 30% to 20%, but it was not statistically significant (p=n.s.). OGTT was normal in 32 patients (80%). The pretreatment impaired glucose tolerance of one patient regressed to fasting glucose intolerance (2.5%). Fasting glucose intolerance improved after therapy in another patient. Hypoglycemia

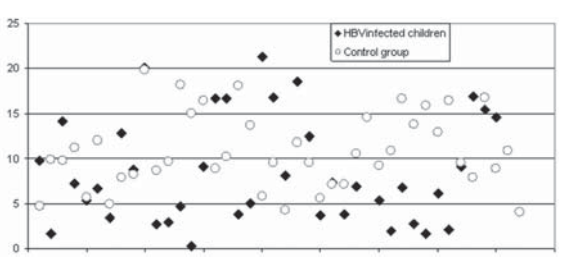

Fig. 2. HOMA-IR levels in CHB-infected children and controls.

Table II. Prevalence of Glucose Metabolism Abnormalities in Children with CHB Before and After Interferon-α Treatment

	Before treatment (n=40)	After treatment (n=40)	p
Frequency of normal glucose tolerance (n, %)	28 (70)	32 (80)	n.s.
Frequency of fasting glucose intolerance (n, %)	1 (2.5)	1 (2.5)	n.s.
Frequency of glucose intolerance (n, %)	1 (2.5)	-	n.s.
Frequency of diabetes mellitus (n, %)	-	-	
Frequency of hypoglycemia (n, %)	10 (25)	7 (17.5)	n.s.
Frequency of FPI>20 μIU/ml (n, %)	2 (5)	2 (5)	n.s.
Frequency of high HOMA-IR (>3.5) (n, %)	7 (17.5)	3 (7.5)	n.s.
Frequency of G/I <6 (n, %)	9 (22.5)	9 (22.5)	n.s.
Frequency of low FPI (<4.1 μIU/ml) (n, %)	13 (32.5)	8 (20)	n.s.
Frequency of low HOMA-cell (<40) (n, %)	9 (22.5)	9 (22.5)	n.s.

n.s.: Non-significant. FPI: Fasting plasma insulin. HOMA-IR: Homeostasis model assessment of insulin resistance. **G/I:** Glucose/insulin ratio.

was present in 7 patients (17.5%). Four of them had mild hypoglycemia. Two children had high insulin levels (FPI >20 μIU/ml). In addition, FPI level of 2 patients was 19 mIU/ml, which is borderline. Eight patients (20%) had lower FPI than the minimum level of the control group. Fasting C-peptide levels were abnormal in 3 patients. Two children had low fasting C-peptide level. HOMA-IR was >3.5 in 3 patients (7.5%). G/I was <6 in 9 patients (22.5%). HOMA-IR was suppressed in 9 patients (22.5%), and G/I was high in 8 patients (20%), enough to indicate increased sensitivity compared with minimum HOMA-IR and maximum G/I levels of the control group (Table II). Nine patients had lower HOMA-cell than minimum HOMA-cell level of the control group. In one patient, anti-GAD positivity (2.5%) was detected after treatment. The GTT results of this patient were normal and there was no increase in the IR or sensitivity. HLA typing of this patient was HLA A2, B5, B51, BW1, BW4, DR11, DR14, DR52, DQ5 and DQ7.

At the end of therapy, complete response was achieved in 3 (7.5%). Two of them had normal OGTT both before and after IFN therapy, while the other responder had hypoglycemia before IFN therapy and normal glucose level after therapy.

Discussion

An altered glucose metabolism had been reported in patients with chronic liver disease and liver cirrhosis. The incidence and severity correlated with the severity and stage of the liver disease (15). The majority of these studies were performed in adult patients with chronic HCV, and the close relationship between DM and chronic HCV infection was reported[2,3,29-31]. However, the exact pathogenesis remains unclear. It is suggested to be a consequence of a number of varied mechanisms that include hyperinsulinemia, IR, reduced hepatic uptake of glucose, and increased sensitivity of β cells to glucose[2,32-34]. In these patients, hyperinsulinemia was shown to be due to decreased insulin catabolism rather than the increase in insulin secretion in the pancreas[2].

Of particular interest, pretreatment FPG and HOMA-IR levels in our patients with CHB were lower than in the control group. In our study, a strong correlation between the degree of liver disease and FPG level was observed, as in Chehadeh et al.'s report[35]. They reported that the prevalence of glucose abnormalities in chronic hepatitis C was significantly higher in patients with liver fibrosis than in patients with no evidence of liver fibrosis or steatosis.

In our study, metabolic evaluations have shown that HBV-infected children had insulin sensitivity, and some of them had lower FPI and HOMA-cell levels compared with controls, features compatible with β-cell hypofunction and impaired insulin secretion. Except for four children, the study population consisted of IFN-naive patients, eliminating the possibility of glucose abnormality related to previous IFN use. Although chronic viral hepatitis has

Table III. Characteristics of Glucose Metabolism Before and After Interferon-α Treatment in Children with CHB

	Before treatment	After treatment	p
Plasma fasting glucose (mg/dl)	76.1±17.1	76.4±11.8	n.s.
Plasma glucose 30th min (mg/dl)	133.3±32.1	115.1±22.1	0.001
Plasma glucose 60th min (mg/dl)	114.3±33.6	97.7±29.2	0.001
Plasma glucose 120th min (mg/dl)	99.6±25.2	85±21.2	0.001
Fasting insulin (μIU/ml)	8.9±6.2	9.3±7.7	n.s.
Insulin 120th min (μIU/ml)	38.5±58.7	23.4±21.2	n.s.
Fasting C-peptide (ng/ml)	3.3±6.1	2.9±1.9	n.s.
HOMA-IR	1.6±1.4	1.7±1.6	n.s.
HOMA-cell	250.6±487.3	245.5±654.8	n.s.
AI antibody positivity (n)	-	-	n.s.
Anti-GAD antibody positivity (n)	-	1	n.s.

Data are expressed as mean±SD
n.s.: Non-significant. HOMA-IR: Homeostasis model assessment of insulin resistance.
AI: Anti-insulin. Anti-GAD: Anti-glutamic acid decarboxylase.

been associated with type 2 DM, there is no evidence to suggest that HBV infection triggers autoimmune destruction of islet β-cells. Indeed, in all of our patients, islet cell antibodies were undetectable. Further studies are needed to elucidate the nature and mechanism of this finding. Before clinical findings emerge in type 1 DM, the first abnormality that could be demonstrated is the loss of acute insulin response during intravenous glucose testing in addition to the presence of autoantibodies[36]. We did not assess the acute insulin response, and this is a limitation of our study.

It is reported that 10% of the patients with chronic hepatitis suffer from disorders of glucose metabolism such as hyperinsulinemia, impaired glucose tolerance and IR[2,37]. High insulin levels and high HOMA-IR (>3.5) were found in only 2 and 7 patients in baseline condition. The few studies investigating the glucose metabolism in adult patients with CHB infection reported that patients with genetic tendency to diabetes may eventually develop IR and diabetes[30,38]. In this study, two children had glucose intolerance before treatment. One of them also had hyperinsulinemia and IR. Except for positive family history of type 2 DM, she did not have any risk factor known to lead to IR. Thus, in the aforementioned patient, the impairment in glucose metabolism was attributed to the patient's genetic tendency for type 2 DM.

The influence of IFN-α on glucose metabolism, development of type 1 DM and changes in diabetes control during its use in patients with CHB have been discussed in only a few papers[6,7,11]. The mechanism of IFN–related DM development or exacerbation of glycemic control remains unclear. Cytokines have an essential role in the destruction of pancreatic β- cells and, consequently, in the pathogenesis of type I DM[39]. IFN is known to induce the immune system and production of free radicals and cytokines, including tumor necrosis factor alpha (TNF-α). Experimental evidence obtained from rat models supports the hypothesis that iatrogenic DM can be induced by maintaining elevated plasma IFN-α levels[40]. IFN-induced experimental DM resulted in intrapancreatic insulitis and consecutive hypoinsulinemia[41]. It is possible that IFN induces autoimmune reaction to pancreas β-cells or destroys pancreas β-cells directly or via free radicals[6-9,14,42,43]. In addition, IFN has been shown to induce IR in the peripheral tissues and in the liver[9]. Both of these mechanisms may be linked to manifestations of glucose intolerance and DM. Contrary to the adverse effects of IFN on glucose metabolism, mentioned above, some studies have shown its positive effect on peripheric insulin sensitivity by clearing HBV and HCV from the liver and improving liver function[11-15,29].

In the present study, we investigated the effect of six months administration of high-dose IFN on glucose metabolism in a larger population. The results are similar to those of our preliminary report and indicate a beneficial effect of IFN-α therapy on glucose metabolism.

Although there was no statistically significant difference, prevalence of glucose abnormalities decreased after IFN administration. Only one patient developed anti-GAD antibody after IFN treatment, suggesting that IFN-α inducing immunological destruction of islet β-cells is unlikely in these patients. In this study, decreased levels of blood glucose in OGTT, regression of glucose intolerance in one patient and improvement in glucose intolerance in another patient after IFN treatment are findings opposing the statement that IFN-α might have adverse effects on glucose metabolism in patients with HBV infection. IFN treatment did not have any positive or negative effect on the β-cell reserve of children in whom low FPI and HOMA-cell levels were present initially. Furthermore, patients who had hyperinsulinemia and IR initially continued to show the same pattern after treatment.

In conclusion, we have established an association between increased insulin sensitivity and CHB in children. Twenty-four percent of them had limited insulin secretory capacity; therefore, they have a risk of developing insulin-dependent DM. Further detailed studies are needed to investigate β-cell reserve in children with CHB. In this small study, no adverse effects of IFN were seen on glucose homeostasis; however, larger studies are required to evaluate this effect more rigorously.

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