

Endocrine Disorders in Pediatric and Adolescent Liver-transplant Recipients

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ABSTRACT

Background: Particular requirements of pediatric and adolescent liver-transplant (LT) recipients necessitate the evaluation of such population from the endocrine viewpoint.

Objective: To determine the endocrine disorders among LT recipients.

Methods: 129 LT recipients younger than 18 years, and at least 6 months post-LT with no previous history of endocrine disorders were included in the study. Demographic, anthropometric and biochemical data were collected.

Results: 36% of cases had evidence of impaired fasting glucose; the problem, however, was dramatically resolved (decreased to 2.3%) by using of prediabetic diet. Identifying only 1 case of primary hypothyroidism indicated that thyroid dysfunction seems not to be a prevalent finding in the patients. 3 cases of rickets and no case of parathyroid dysfunction were identified. 11% of the study population were hypocalcemic (2 had rickets as well). Pubertal condition in 3 patients and delayed puberty before LT in 6 remained the same; further evaluation revealed they had hypogonadotropic hypogonadism.

Conclusion: Regular monitoring for development of diabetes and hypocalcemia is indicated. Evaluation of those with delayed puberty for receiving sexual hormones is also recommended.

KEYWORDS: Liver transplantation; Endocrine system; Diabetes mellitus; Thyroid diseases

INTRODUCTION

Liver transplantation (LT) in pediatrics and adolescents affects not only the social and psychological features but also the physical aspects of general well-being [1]. Among the latter are different endocrine disorders that are prevalent in children, and in turn, can complicate the clinical condition of the patients.

A great body of literature exists concerning the common prevalence of post-transplant

metabolic syndrome (MetS); its individual components, and the potential risk factors in adult population [2-7]. Although its etiology is multifactorial, MetS can increase the risk of major vascular complications and non-alcoholic fatty liver disease (NAFLD) [3, 7, 8]. Post-transplant diabetes mellitus (PTDM), as a MetS constituent, is an iatrogenic diabetes, the exact etiology of which is still unknown [9]. The subsequent consequences of pediatric PTDM are thought to be the usual diabetic complications together with those associated with the immune suppression [9]. It is, however, postulated that the condition can improve through lifestyle modification including dietary alteration, physical activity, and weight control [2, 6, 10]. Other aspects of the endocrine dysfunction, such as thyroid or para-

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thyroid dysfunction, and disorders of mineral homeostasis were rarely studied, even in adult population [11].

Due to the importance of the issue and limited evidence comprehensively considering various aspects of the endocrine disorders in pediatric LT-recipients population, the current prospective study was conducted to concurrently evaluate various endocrine disorders including PTDM, thyroid and parathyroid dysfunction, disorders of mineral homeostasis, and pubertal development.

PATIENTS AND METHODS

A total of 129 pediatric and adolescent LT recipients with no previous history of endocrine disorders in themselves and their first-degree family was evaluated prospectively for subsequent development of such diseases between November 2015 and December 2016. The patients aged <18 years, and were at least six months post-LT at the time the study was initiated. Demographic, anthropometric, and biochemical data of the study population were collected. Underlying cause(s) of liver disease, relevant prescribed drugs, pre- and post-operative clinical history, and immunosuppressive regimen of the patients were recorded as well. Signed informed consents were provided by patient's parents as well.

The patient's height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively, to calculate the body mass index (BMI). Fasting venous blood samples were drawn from all the patients to measure (1) fasting blood sugar (FBS, mg/dL), and glycosylated hemoglobin (Hb A1c; %) to determine the cases with post-LT IFG or diabetes mellitus (DM); (2) thyrotropin/thyroid stimulating hormone (TSH; $\mu\text{g/mL}$), total thyroxine (TT4; $\mu\text{g/dL}$), total tri-iodothyronine (TT3; ng/dL), and tri-iodothyronine resin uptake (T3RU; %) to screen the study population for any evidence of thyroid dysfunction, since the exact measurement of free T4 could not be performed using available kits in our center; (3) calcium (Ca; mg/dL); phosphorus (P; mg/dL), parathyroid hor-

mone (PTH; pg/mL), alkaline phosphatase (ALP; U/L), 25-OH vitamin D (25-OH D; ng/mL), and albumin (Alb; g/dL) to identify any possible case of rickets or parathyroid disorders; and (4) luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol or testosterone for those patients with the history of delayed puberty before LT.

Serum concentrations of FBS, Ca, P, ALP, and Alb were measured using a colorimetric spectrophotometry method (Biosystems SA, Barcelona, Spain). Thyrotropin, LH, and FSH levels were determined by immunoradiometric assay (IRMA) (Padyab Teb, Alborz, Iran). Measurement of TT4, TT3, T3RU, and testosterone was measured by radioimmunoassay (RIA) kits (Padyab Teb, Alborz, Iran; IZOTOP, Europe) according to the manufacturer's instructions. PTH was determined using a sandwich immunoassay (Cobas; Roche Diagnostics, Mannheim, Germany). Hb A1c was measured by high-performance liquid chromatography (HPLC) method (Hewlett Packard 1100 USA).

Normal glucose metabolism was defined as an FBS of <99 mg/dL. IFG was defined as the presence of FBS of 100–125 mg/dL. The glycemic control of pediatric and adolescent LT-recipients was initially evaluated by measurement of FBS. Patients with FBS of 100 mg/dL or more were re-evaluated on the basis of serum FBS, and Hb A1c following adherence to a 3-month-prediabetic diet.

Ca, P, ALP, and Alb were measured in the serum; 25-OH D and PTH were measured in those with abnormal values to determine the possible parathyroid disorders or rickets.

Pubertal development was assessed by a researcher of the same sex as the patients. Delayed puberty was defined as the absence of any signs of puberty in girls of 13 and boys of 14 years of age or older based on the Tanner criteria.

Since medical nutrition therapy (MNT) is considered the cornerstone of diabetes care and management, prediabetic regimen of ad-

equate healthy foods, based on 2016 American Diabetic Association (ADA) recommendations, was taken as the first-choice treatment. The main emphasis of the prescribed regimen was placed on portion control, use of whole grains, vegetables, fruits, legumes, and low-fat dairy products such as carbohydrate sources with focus on foods and/or beverages with higher fiber content, lower glycemic load, and less sugar-sweetener. Eating antioxidant-rich foods (*e.g.*, those containing vitamins E, C and carotenoids) and dietary sources of long-chain omega-3 and monounsaturated fatty acids, such as fatty fish, nuts, seeds and olives was also recommended [12].

Statistical Analysis

IBM SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data analysis. Categorical and continuous variables were presented as number (together with percentage), and mean±SD, respectively. Pearson χ^2 and Fisher's exact test (when applicable) were used to compare the categorical variables.

RESULTS

A total of 129 (70 [54.3%] male and 59 [45.7%] female) LT recipients with a mean±SD age of 10.7±4.3 years were examined. No significant sex difference was observed ($p=0.33$). Of the study population, 72 (55.8%) were young (>10 years) and 13 (10.7%) were child (<5 years). The mean±SD time since LT was 3.2±2.1 years. The mean±SD height-for-age Z score was -1.2±1.6, which had a significant ($p=0.01$) positive correlation with age ($r=0.26$). No relationship was observed between the height-for-age Z score and sex. Almost 86% of the cases had a BMI <25 kg/m² (Table 1). No transplant rejection occurred during the course of the study. Twenty percent of the survey subjects experienced only one, and approximately 5% had more rejection episodes in their past history. Graft function (assessed by periodic laboratory tests) was good while the study was run.

The underlying etiologies for LT were mainly biliary atresia ($n=28$), Wilson's disease ($n=18$),

Table 1: General characteristics of LT recipients ($n=129$). Values are either n (%) or mean±SD.

Parameter	Statistics
Sex (Male)	70 (54.3%)
Age, yrs	10.7±4.3
Boys	9.8±4.0
Girls	11.8±4.5
Time elapsed since LT, yrs	3.2±2.1
BMI, kg/m ²	21.8±3.4
Underweight (<17)	53 (41.0%)
Normal weight (17-24.9)	58 (45.0%)
Overweight (25-29.9)	10 (7.7%)
Obese (≥30)	8 (6.3%)
Height-for-age Z score	-1.2±1.6
Corticosteroid	11 (8.5%)
Tacrolimus	129 (100%)

progressive familial intrahepatic cholestasis (PFIC) ($n=17$), autoimmune hepatitis (AIH) ($n=14$), cryptogenic cirrhosis ($n=13$), Crigler-Najjar syndrome ($n=13$), and hypercholesterolemia ($n=10$). Other less common causes included neonatal hepatitis, tyrosinemia, idiopathic neonatal hepatitis (INH), hepatocellular carcinoma (HCC), choledochal cyst, glycogen storage disease (GSD), and hepatitis B, which totally accounted for 23 cases.

Immunosuppressive regimen after LT included pulse methylprednisolone for the first 3 days that then changed to oral prednisolone 1 mg/kg (Max 20 mg daily), which tapered to discontinue during 3–6 months after liver transplantation, except in cases with AIH. The regimen was followed by tacrolimus (FK-506; Prograf) to obtain a serum level of 7–10 ng/mL in the first year and 5–7 thereafter, and prednisolone (1 mg/kg) which gradually tapered down aiming to complete steroid withdrawal by the end of the sixth post-LT month except for those with acute rejection or elevated liver enzymes. In the case of inappropriate response to FK, Cellcept was added to the immunosuppressive regimen at a dose of 20–40 mg/kg/d.

Eighty-two (63.6%) patients had normal glucose metabolism in the post-LT period. Of the remaining 47 recipients, 44 cases with IFG

(mean FBS of 109 mg/dL) and three with an FBS of >126 mg/dL (129, 131 and 134), were also identified. Of those being nutritionally managed with a 3-month prediabetic regimen, more than 93% were treated. Among them, there were three previously mentioned cases with FBS >126 mg/dL whose FBS and Hb A1c reached the acceptable levels of <99 mg/dL and <5.7%, respectively, after the nutritional intervention. IFG concentration of LT recipients had no relationship with FK-levels in those three patients with abnormal Hb A1c values; only one was on Cellcept medication, as well. IFG persisted in 3 (7%) of 47 patients in spite of prediabetic diet.

No relationship was found between the transplant type (partial *vs* whole organ), sex or age of the study population, Cellcept therapy, and FK levels and glucose level of IFG patients in the first screening round.

Increased serum TSH levels (for age) were identified in six LT recipients with abnormal thyroid function. Repeating the measurements of TSH and TT₄, however, identified only one with primary hypothyroidism with a TSH >50 µg/mL. The patient was a 6-year-old boy with a height-for-age Z score of -3.67 and BMI of 15.3 kg/m² who had undergone a whole organ transplant as the result of HCC occurred in the background of tyrosinemia a year before the survey. He had a normal FK level and was on glucocorticoid medications, but not Cellcept.

Evaluation of Ca, and P revealed two cases of hyperphosphatemia. However, due to normal PTH, ALP, and Alb levels, hypoparathyroidism was ruled out in both. No case of hypo- or hyperparathyroidism was detected. About 11% of the study population was hypocalcemic with normal serum levels of P, ALP, Alb, 25-OH D, and PTH who were put on the standard treatment regime of divided doses of Ca-glibionate 50 mg/kg/day together with 400 IU daily supplementary vitamin D. No association was also detected among serum Ca and age, sex, transplant type, BMI, and the administered drugs.

Fourteen cases of low Ca with normal or lower limit of normal serum P levels were identified; two had high ALP levels (for age). Both were boys with lower limit of normal serum P levels and low 25-OH D level who were put on medication of 300,000–600,000 IU vitamin D (solved in 5 mL olive oil and administered orally at a dose of 1 mL/h for 6 hours) in combination with daily dose of 400–800 mg Ca and regular endocrinologist follow-up for rickets.

A case of biochemical rickets was also detected and put on medication. The patient was a 17-year-old girl with a BMI of 25 kg/m² who had received whole organ LT due to autoimmune hepatitis (AIH) almost two years before the study. She had a serum Ca level of 7.6 mg/dL, P of 2.6 mg/dL, and Alb of 4.1 g/dL. Serum concentrations of ALP, and 25-OH D were 240 U/L, and 12 ng/mL, respectively.

Pubertal development of 19 patients was assessed before LT. Of those six had delayed puberty at the first examination; the pubertal condition remained the same in only three patients—a boy and a girl both aged 17 who had undergone whole organ LT due to Wilson's disease and hypercholesterolemia, respectively, both with a history of rejection; and a 16-year-old boy who had undergone partial transplant as a result of tyrosinemia. Further evaluation revealed that they had hypogonadotropic hypogonadism. Sex hormone replacement therapy was started. The other three girls experiencing delayed puberty before the LT, had had either choledochal cyst (1 case) or AIH (2 cases). No correlation was found between height-for-age Z score and BMI and any of endocrine disorders.

DISCUSSION

The main finding of the current study was that 36% of cases had evidence of IFG. The frequency of impaired glucose metabolism in the study population was well comparable to the overall incidence reported for orthotopic LT adults by Tueche, *et al* (31%), and Soule, *et al* (33%); lower values have been described

Table 2: The prevalence of PTDM reported by other investigators

City, Country	LT-reipient (age)	Prevalence/incidence (%)	Other findings	Reference
France	OLT adult (21–68 yrs)	31 (60% pre-LTDM; 40% <i>de novo</i> PTDM)	Introduction of pre-OLT DM, alcoholic cirrhosis, and male sex as predictive factors for PTDM. No association between PTDM and HCV infection.	13
France	Adults (–)	22.7	Introduction of maximum lifetime BMI, IFG, and HCV status as relevant PTDM factors	27
Japan	Adults (–)	49.6	No association between NODM and HCV	23
China	Adults (>18 yrs)	25	Introduction of BMI ≥ 25 kg/m ² before LT as the only independent risk factor for PTDM	24
Brazil	Adults (≥ 18 yrs)	18.3	Found an association between PTDM and HCV infection	15
Italy	Adults (–)	10.8	More incidence of NODM in patients >45 yrs and/or HCV-positive	16
USA	OLT Adult (>18 yrs)	33	Introduction of HCV, male sex, and age >50 yrs as independent risk factors for development of NODM	14
USA	Adults (–)	17.8	Introduction of sex as the only independent risk factor for development of NODM	28
USA	Pediatrics	13	Increased incidence of early GI due to age >5 yrs at the time of transplant, hospitalization at transplant, early steroid use, and tacrolimus use	9

LT, liver transplant; PTDM: post-LT diabetes mellitus; GI: glucose intolerance; OLT: orthotopic LT; DM: diabetes mellitus; NODM: new-onset DM; HCV: hepatitis C virus; BMI: body mass index

for either pediatric or adult patients by other researchers—Hathout, *et al* (13%), Parolin, *et al* (18.3%), and Mirabella, *et al* (10.8%) [9, 13–16]. This can be at least partially attributed to various definitions of the new-onset DM and the time elapsed since the operation. The problem, however, was mostly resolved (to 2.3%) through the application of a 3-month prediabetic diet.

PTDM is considered a progressive complication of organ transplantation [9], the precise etiology of which is not yet recognized. It, however, appears to be attributed to β -cell toxicity and increased insulin resistance caused by diabetogenic immunosuppressive regimens [17–22]. The most available studies evaluating the frequency of either GI or DM after LT were conducted on adult population [13, 15, 23, 24]. However, only one study has so far investigated the issue in pediatrics and/or adolescents undergoing LT (Table 2) [9].

Totally, male sex, age >45 years, overweight or obesity and probably hepatitis C virus (HCV) infection have previously been reported to be the independent risk factors for the development of PTDM [13–15, 24, 25]. In the current study, no association was found between IFG and sex of participants. This finding was not consistent with those of Tueche, *et al*, and Soule, *et al*, who report that the incidence of PTDM is higher in males [13, 14]. Moreover, although several studies, both in pediatric or adult patients, have confirmed the relationship between PTDM and age of the population [9, 14, 16], no such association was found in our study.

BMI of the study participants did not have any association with impaired glucose metabolism. This was in accordance with those of Hathout, *et al* [9]. It is, however, inconsistent with those of Zhao, *et al* [24], who report that BMI ≥ 25 kg/m² is associated with the development of PTDM.

The height-for-age Z score had a positive correlation with age ($r=0.26$, $p=0.01$). This indicated that younger children who had been operated due to the severity of the disease and their growth pattern was influenced more than the older children and adolescents, who had undergone LT at higher ages due to the acute disease. The latter group had had a better growth pattern before LT or had been transplanted many years ago when they were very young, so they had had a suitable growth pattern due to the successful transplantation.

Identifying only one patient with hypothyroidism indicated that thyroid dysfunction seems not to be a prevalent finding in the patients; no similar study was found to compare the present results with its findings.

About 10% of the patients were hypocalcemic with normal values of other relevant parameters. Impaired vitamin D metabolism as well as glucocorticoid/immunosuppressive use can be suggested as the underlying mechanisms.

Hypothalamic-pituitary gonadal (HPG) axis function is related to various factors. The more chronic the underlying cause of LT, the more impaired the function of axis. Delayed puberty in LT recipients receiving immunosuppressive and steroids, seems to be at least partially due to decreased nocturnal pulsatile release of the growth hormone and gonadotropins [26]. It is, therefore, recommended that the patients should be evaluated for receiving sexual hormones.

In conclusion, considering the dramatic decrease of IFG in pediatric or adolescent LT recipients following administration of a 3-month prediabetic diet, it seems that regular post-operative monitoring of FBS, Hb A1c and if possible oral glucose tolerance test (OGTT) once or twice a year is mandatory, for early detection and prevention of DM and to provide good long-term results through prediabetic regimen and lifestyle modification. Thyroid disorder was not a common finding in our study population. Annual screening for rickets and hypocalcemia is recommended as well. Delayed puberty should also be evaluated in

routine physical examination.

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