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Nucleos(t)ide analogues for the prevention of hepatitis B recurrence after liver transplantation do not affect serum phosphorus levels

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Abstract

Background Nucleos(t)ide analogues (NAs) constitute the backbone of treatment for the prevention of hepatitis B virus recurrence after liver transplantation (LT). Decline in serum phosphorus levels is a common side effect of nucleotide therapy. Our aim was to assess the impact of nucleotide treatment on the occurrence of hypophosphatemia after LT and determine possible predictors.

Methods We retrospectively analyzed data from liver transplant recipients who had been transplanted for various indications. All patients were evaluated every 3 months. Each patient was considered to be having hypophosphatemia when at least one value of serum phosphorus ≤2.5 mg/dL was detected.

Results In total, 109 patients [83 males (76%)] with a mean age of 55±10 years were included. 46/67 (67%) patients with hepatitis B received a nucleotide. The rate of hypophosphatemia (55%) was not different between patients with hepatitis B and those transplanted for other indications (62%). Patients receiving a nucleotide did not run a greater risk of hypophosphatemia than patients receiving only nucleosides (59% vs. 48%, P=0.39). Male gender and everolimus use were associated with the occurrence of hypophosphatemia in patients with hepatitis B. In multivariate analysis only gender was associated with hypophosphatemia (odds ratio 11.43, 95%CI -2.11 to -0.49; P=0.0025).

Conclusions Hypophosphatemia occurs in more than half of liver transplant recipients regardless of the indication for LT. Male gender and everolimus use seem to predispose to hypophosphatemia, whereas the type of antiviral agent does not.

Keywords Hepatitis B, hypophosphatemia, nucleos(t)ide, prophylaxis, transplantation

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Introduction

The combination of hepatitis B immunoglobulin (HBIG) with a potent nucleos(t)ide analogue (NA) - namely entecavir (ETV) or tenofovir (TDF) - is currently considered the standard of care for prophylaxis against recurrence of hepatitis B virus (HBV) after liver transplantation (LT) [1]. This regimen has been adopted by the majority of liver transplant centers, although the optimal treatment schedule remains to be elucidated. Nonetheless, this approach has been proven very effective and up to this point no serious safety signals have appeared in this population.

Nucleotides (adefovir, ADV and TDF) have been reported to cause hypophosphatemia (low phosphorus serum levels). This was initially noted with the use of ADV, especially in solid organ transplant recipients [2,3]. Subsequently, a decrease in phosphorus levels was considered to be a common side effect of TDF therapy as well, although the registration trials of this drug had shown no significant rates of hypophosphatemia [4,5]. The mechanism for this adverse event is thought to be through proximal renal tubular damage leading to loss of reabsorption capability of phosphorus, amino acids and glucose [6]. Renal impairment is associated with increased incidence of this side effect.

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Conflict of Interest: None

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The majority of liver transplant recipients gradually develop chronic kidney disease [7]. Theoretically, this population runs a greater risk of developing renal side effects, including hypophosphatemia. Nucleotide treatment could enhance this adverse event along with other predisposing factors for kidney disease that are usually prevalent in these patients (e.g. hypertension and diabetes mellitus). However, there are very few data available regarding serum phosphorus levels in liver transplant recipients receiving nucleotide treatment, thus leaving this important clinical issue still unanswered. In the present study we aimed to assess the impact of nucleotide treatment on the occurrence of hypophosphatemia and to determine the clinical significance and predictors of hypophosphatemia in liver transplant patients.

Patients and methods

Study population

We retrospectively analyzed data from liver transplant recipients followed in the liver transplant center in Thessaloniki, Greece from May, 1993 to January, 2010. All patients in our center received cadaveric transplants. They were evaluated routinely every 3 months. Patients were divided in two groups. The first included patients with advanced liver disease due to HBV infection as the primary indication for LT and the second patients with advanced liver disease due to hepatitis C or alcoholic liver disease as the primary indication for LT. Co-infection with delta virus or associated hepatocellular carcinoma at the time of LT were not considered as exclusion criteria. All patients with HBV infection received treatment with NAs (either as prophylaxis or treatment of HBV infection). The minimum follow up was 6 months. Once the specific study population was defined, a retrospective chart review was performed in order to retrieve the demographic and laboratory data of these patients. In addition, information on immunosuppressive regimens and concomitant diseases was followed. The study was approved by our institutional review board.

Laboratories/definitions

Routine laboratories were used to calculate biochemical parameters. Phosphorus levels in particular were routinely evaluated at every visit. The MDRD (Modification of Diet in Renal Disease) formula was used to determine creatinine clearance [8]. Hypophosphatemia was defined as a value of serum phosphorus ≤2.5 mg/dL. Hypophosphatemia was considered to be persistent when it was noted in at least 3 consecutive evaluations.

Antiviral treatment

NAs were prescribed at recommended doses according to published guidelines. Based on the time period of the examination, drug availability and policy of the transplant center, both nucleosides (lamivudine and ETV) and nucleotides (ADV and TDF) were used.

Statistical analysis

Continuous variables were presented as means ± standard deviation or median. Comparisons between the two groups were performed using independent t-tests if values were normally distributed or by the Wilcoxon rank sum test if the distribution was not normal. Frequency data were presented as number and percentage and compared using the chi-square test or Fisher's exact test when appropriate. Baseline laboratory values with a significance of $P \leq 0.1$ were entered in a multivariate model of multiple linear regression analysis to explore possible correlations of hypophosphatemia with the baseline characteristics.

Results

One hundred and nine patients were included in this study. Table 1 summarizes the basic demographic and laboratory characteristics of the patients. No patient was found to have human immunodeficiency virus (HIV) co-infection. Nearly all patients with HBV infection (93%) received the NA treatment as prophylaxis against recurrence of HBV. A nucleotide was included in the NA regimen in two thirds of the patients (Fig. 1).

Occurrence of hypophosphatemia

More than half of the patients in the total cohort (63/109, 58%) were diagnosed with hypophosphatemia during the follow-up period. No significant difference in the occurrence of hypophosphatemia was noted between the two groups of patients (P=0.49). The median phosphorus value designating hypophosphatemia was 2.1 mg/dL (range 1.2–2.4), whereas 19/63 patients with hypophosphatemia had levels of phosphorus below 2 mg/dL. Hypophosphatemia was persistent in 51% (32/63) of the patients. Of note, 12/109 could not be assessed for having persistent hypophosphatemia as low phosphorus levels were detected during their last or penultimate visit.

The effect of nucleotide treatment

Among patients with hepatitis B, patients under NA regimens including a nucleotide did not have a greater risk of hypophosphatemia compared to patients who were receiving only nucleosides (59% vs. 48%, P=0.39) (Fig. 2). Moreover, the time interval between the occurrence of hypophosphatemia and NA initiation did not differ significantly between

Table 1 Patients' demographic and laboratory values

	Total cohort	Patients with hepatitis B	Patients with hepatitis C/ alcoholic liver disease	P
N	109	67	42	
Age, years	54.9±9.9	53.3±11.1	57.6±6.9	0.06
Male gender, n (%)	83 (76)	49 (73)	34 (81)	0.35
Baseline phosphorus*, mg/dL	3.47±0.76	3.34±0.65	3.66±0.87	0.004
Baseline creatinine clearance**, mL/min	81.5±30.2	82.6±30.4	79.8±30	0.69
Hypertension, n (%)	30 (28)	23 (35)	7 (17)	0.03
Diabetes, n (%)	18 (17)	11 (17)	7 (17)	>0.99
Calicineurin inhibitor use, n (%)	99 (92)	59 (89)	40 (95)	0.28
Mycophenolate mofetil use, n (%)	89 (82)	51 (77)	38 (90)	0.07
Everolimus use, n (%)	32 (30)	19 (29)	13 (31)	0.81

Data are presented as mean±standard deviation unless otherwise indicated. *One month post-transplant. **As calculated with the MDRD (modification of diet in renal disease) formula one month post-transplant

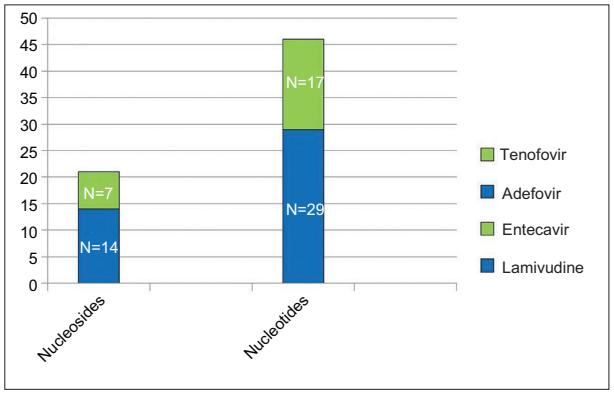

Figure 1 Distribution of nucleos(t)ide analogues that were used in patients transplanted for advanced liver disease due to chronic hepatitis B

the two groups (1.6 vs. 2 years, P=0.19). In addition, the creatinine clearance at the time of hypophosphatemia was not significantly different for patients receiving nucleotides as compared with those receiving only nucleosides (69.1 vs. 81.3 mL/min, P=0.09).

The mean time period between initiation of the NA and occurrence of hypophosphatemia was 1.7±1.4 years. However, only one patient -receiving ADV- had symptoms related to hypophosphatemia (muscle weakness), which quickly resolved after switching to a nucleoside.

Predictors of hypophosphatemia in patients with hepatitis B

In univariate analysis male gender and everolimus use were associated with the occurrence of hypophosphatemia (Table 2). Notably, patients with hypophosphatemia did not have lower creatinine clearance at the time of hypophosphatemia as compared with patients without hypophosphatemia at the time of their last follow-up visit (72.5 vs. 72.7 mL/min, P=0.92).

Table 2 Predictive factors for hypophosphatemia

	OR	CI (95%)	P-value
Male gender	7.21	2.04-25.48	0.001
Everolimus use	4.26	1.22-14.76	0.017

OR, odds ratio; CI, confidence interval

However, patients with hypophosphatemia seemed to be having a deterioration of their creatinine clearance, as expressed by the difference in creatinine clearance values, compared with patients without hypophosphatemia (7 vs. -1.5 mL/min, P=0.09). No specific combination of immunosuppressants was a predictive factor for hypophosphatemia. Finally, everolimus use was associated with persistent hypophosphatemia (63% vs. 17%, P=0.004).

In multivariate analysis only gender was associated with the occurrence of hypophosphatemia (odds ratio 11.43, 95%CI -2.11 to -0.49; P=0.0025). Notably, male gender was the only predictor of hypophosphatemia -both in univariate and multivariate analysis- in the total cohort of patients, as well.

Discussion

Currently, prophylaxis against recurrence of HBV after LT is based on treatment regimens including an NA. Although the additional use of HBIG in the regimen remains equivocal, NAs undoubtedly serve as the backbone for treatment in this setting [9]. Nucleotides, in particular, have been initially reported to cause hypophosphatemia [2,3]. Several case reports in the literature have confirmed this initial observation providing adequate evidence for the occurrence of this adverse event in the pre-transplant setting [10-15]. Remarkably, hypophosphatemia was recently shown to occur in up to 35% of patients with hepatitis B receiving ADV after a mean treatment period of 57 months [16]. However, since no major clinical sequelae have been recorded along

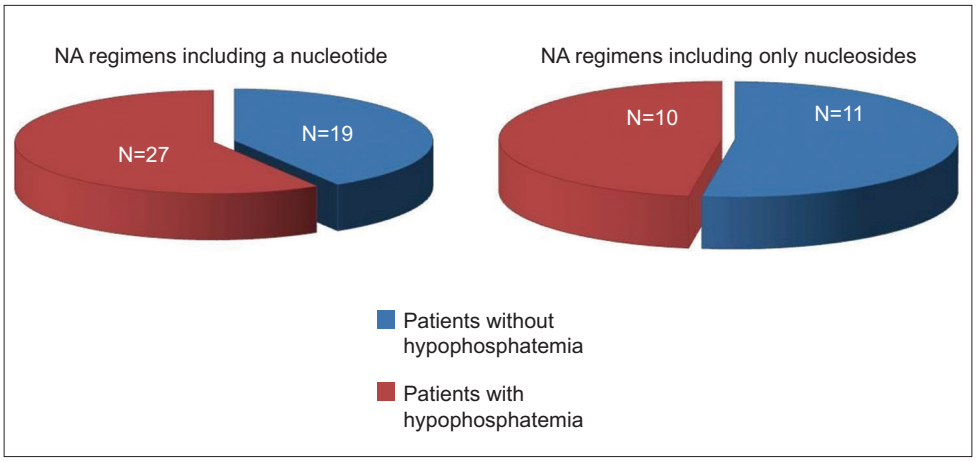

Figure 2 Occurrence of hypophosphatemia according to nucleos(t)ide analogue regimen

with the occurrence of hypophosphatemia, monitoring for this adverse event has become rather vague after LT [17,18]. A preliminary report in kidney recipients has shown no changes in serum phosphorus levels under ADV therapy [19]. Nonetheless, case reports in post transplant patients with hypophosphatemia have been recently published, as well [20]. To our knowledge no large scale information has been published on the prevalence and significance of this side effect in liver transplant recipients. In response to this lack of data, we report in this study a significant (55%) rate of hypophosphatemia occurrence in liver transplant recipients receiving NA for at least 1.7 years.

Hypophosphatemia is thought to be part of the well characterized harmful effect of nucleotides on renal function. This effect is shown to be dose-dependent and is more pronounced with the use of ADV than TDF [21]. The reduction in serum phosphate levels in patients under nucleotide treatment is typically noticed after months or even years of treatment. In general, it is caused by a direct toxic injury of the drugs at the proximal renal tubules leading to a syndrome that resembles Fanconi's syndrome with hypophosphatemia, hypouricemia, aminoaciduria and glucosuria [6]. Uptake of ADV into the proximal tubular cells is mediated by the human organic anion transporter-1 on the basolateral membrane, whereas secretion is via the apical multidrug resistant protein-4. Overexpression of the former or underexpression of the latter may increase exposure of proximal tubular cells to ADV. Depletion of mitochondrial DNA from the renal tubular epithelium may also contribute to the pathophysiology of this injury.

In this setting, hypophosphatemia may also lead to the development of osteomalacia due to inadequate mineralization of the bone matrix [22]. Hydroxylation of vitamin D occurs primarily in the proximal tubules, whereas parathyroid hormone (PTH) was recently shown to increase in patients infected with HIV that were receiving TDF [23]. The most common symptoms include nonspecific diffuse bone pain and polyarthralgia, which make diagnosis of this condition difficult unless there is a high index of suspicion. In our population, only one patient reported muscle weakness making hypophosphatemia a laboratory abnormality with no clinical sequelae, although a significant portion of patients had levels below 2 mg/dL.

In the most elaborate description of renal tubular dysfunction this condition was found in 14% (7/51) of patients with hepatitis B receiving nucleotides - mainly ADV [24]. In this study, serum phosphorus value had to be persistently below 2.5 mg/dL and at the same time lower by at least 0.5 mg/dL from the baseline value to denote hypophosphatemia. In addition, two other features of tubular dysfunction -hypouricemia, proteinuria, glucosuria, sustained creatinine increase- had to be present. Several other patients had some of these laboratory abnormalities, but failed to meet the definition for tubular dysfunction. Similar findings were also reported with the use of TDF in patients infected with HIV [25]. The greater occurrence rate of hypophosphatemia in our population could be explained by the fact that we determined phosphorus levels as a sole biochemical parameter and did not set strict criteria for the definition of tubular dysfunction. This approach was elected as the nature of the study (retrospective) did not allow access to the complete set of laboratory data required to study the kidney tubular function of our patients. Another important point that one has to take in consideration is the fact that our study was performed in liver transplant recipients and not patients with chronic hepatitis B. Theoretically, this population runs a greater risk of developing renal side effects as the majority of liver transplant recipients gradually develop chronic kidney disease [7]. The effect of immunosuppressives on renal function and the augmented rate of arterial hypertension and diabetes mellitus post LT could also play a role in the occurrence of hypophosphatemia.

In this study, we unexpectedly found that the choice of NA (nucleoside vs. nucleotide) did not affect the occurrence of hypophosphatemia. The percentage of patients with hypophosphatemia was greater among patients receiving nucleotides as compared with those receiving nucleosides (59% vs. 48%). However, this difference did not reach statistical significance possibly due to the relatively small sample size of our study. Notably, no difference in the levels

of serum phosphorus was also found in another study of our center evaluating monoprophylaxis against HBV recurrence with ETV or TDF [26]. Nucleoside analogues are not known to have renal toxicity. This fact raises the possibility that other factors, apart from the choice of NA, play a role in the occurrence of hypophosphatemia in liver transplant recipients. This is also underlined by the finding that the rate of hypophosphatemia did not differ between patients that were transplanted for hepatitis B as compared to patients that were transplanted for hepatitis C or alcoholic liver disease.

Male gender and inclusion of everolimus in the immunosuppressive regimen were found to increase the likelihood of hypophosphatemia in our population. As expected, the decline in creatinine clearance seemed also to predispose to low phosphorus values. In general, hypophosphatemia can be related to decreased intestinal absorption of phosphorus, redistribution of phosphorus from the extracellular to the intracellular compartment, increased loss of phosphorus through the kidneys, or any combination of these processes [27]. Of note, certain populations are likely to include a greater proportion of hypophosphatemic patients, e.g., alcoholics, septic and malnourished patients. Male gender *per se* has not been described as a predisposing factor for hypophosphatemia so far, although it is conceivable that certain factors (e.g. alcohol consumption) could indirectly influence the occurrence of hypophosphatemia in males. Focused research is warranted to elucidate the mechanisms that relate male gender with the occurrence of hypophosphatemia. Everolimus is an inhibitor of the mammalian target of rapamycin (mTOR-I). mTOR is a regulatory protein kinase involved in lymphocyte proliferation, developmental processes such as neurologic and muscle generation and tumor cell growth. A reduction in phosphate levels is an important toxicity of mTOR-I therapy, but the exact mechanism of this effect is not known [28].

Apart from the retrospective design, there are some other limitations of our study that have to be mentioned. First, the lack of data regarding the serum levels of NA decreases the power of our results as it has been found that tubular dysfunction is associated with higher TDF plasma concentrations [25]. Finally, the detailed evaluation of the skeletal effects of NA entails measurements of vitamin D and PTH that were missing in our study.

In conclusion, hypophosphatemia as a sole biochemical finding occurs in more than half of liver transplant recipients receiving an NA for prevention of hepatitis B recurrence. This adverse event occurs in similar rates in liver transplant recipients with different indications. In this setting, hypophosphatemia seems to be only a laboratory abnormality as no serious clinical sequelae were reported. Interestingly, the choice of a nucleotide instead of a nucleoside was not found to increase the risk of hypophosphatemia in this setting. On the contrary, male gender seems to predispose to hypophosphatemia. Additional prospective studies are warranted to elucidate the effect of NA in renal tubular function and to study the skeletal effects of NA post LT.

Summary Box

What is already known:

- The combination of hepatitis B immunoglobulin with a potent nucleos(t)ide analogue is currently considered the standard of care for prophylaxis against recurrence of hepatitis B virus after liver transplantation
- Nucleotides have been reported to cause hypophosphatemia, but studies are lacking post transplantation
- The majority of liver transplant recipients gradually develop chronic kidney disease, thus increasing their risk for developing renal side effects, including hypophosphatemia

What the new findings are:

- Hypophosphatemia occurs in more than half of liver transplant recipients regardless of the indication for liver transplantation
- The use of a nucleotide instead of a nucleoside does not increase the risk of hypophosphatemia in patients with hepatitis B
- Male gender seems to predispose to hypophosphatemia

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