Persistently Elevated Urine β2-MG and Decreased BMD in a Patient with ADV-induced Hypophosphatemic Osteomalacia and Fanconi's Syndrome After Drug Withdrawal

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Abstract: This is the first case of adefovir dipivoxil (ADV)-induced hypophosphatemic osteomalacia (HO) and Fanconi's syndrome in which bone mineral density (BMD) is continuously reduced after drug discontinuation**.** Sustained elevation of urine β2-MG also supports the recent view that ADV-induced renal injury is not completely reversible.

Keywords: Adefovir dipivoxil, Fanconi's syndrome, Hypophosphatemic osteomalacia, Osteoporosis, Chronic hepatitis B virus, Bone mineral density, β2-microglobulin

Key Clinical Message: This is the first case of ADV-induced hypophosphatemic osteomalacia and Fanconi's syndrome in a female CHB patient in which BMD was consistently reduced after ADV cessation and phosphorus supplementation.

1. INTRODUCTION:

Approximately one third of the world's population has serological evidence of past or present infection with hepatitis B virus (HBV) and more than 350 million people are chronic HBV surface antigen (HBsAg) carriers[1]. Currently there are two therapeutic regimens approved for CHB treatment: immunotherapy and NUCs that can arrest HBV replication[2]. ADV is a diester prodrug of adefovir, an acyclic nucleotide analogue of adenosine monophosphate. Following oral administration, ADV is rapidly converted to adefovir, which is further phosphorylated by cellular kinases to the active metabolite adefovir diphosphate and suppress HBV by selectively inhibiting viral polymerase and reverse transcriptase[3, 4]. Adefovir is renally eliminated through a combination of glomerular filtration and active tubular secretion. The high-dose or long-term ADV administration was associated with renal toxicity, HO and even Fanconi's syndrome. Although it is generally believed that a daily dose of 10 mg is safe, as shown in short-term clinical trials[4], there have been cases of low-dose ADV causing kidney injury and HO in long-term observation[5, 6], and ADV-induced nephrotoxicity could be irreversible in some reports[7, 8].

We present here a case of ADV-induced HO and Fanconi's syndrome, whose clinical symptoms resolved and laboratory abnormalities basically normalized after ADV

discontinuation and phosphorus supplementation. However, urinalysis and DXA, respectively 19 and 70 months after the cessation of ADV, showed persistently elevated β2-MG and decreased BMD. This severe long-term adverse reaction presented in our report calling for detailed bone metabolic investigations and carefully renal function monitor in patients taking ADV.

2. CASE DESCRIPTION:

A 50-year-old Chinese CHB woman who had severe generalized musculoskeletal pain, weakness, and weight loss was referred to the liver disease clinic of our hospital in February 2015. Figure1 fully describes the timeline of her medical history and medication. The patient's mother had hepatitis B, which she acquired through vertical transmission. In May 2005, she had active hepatitis B with elevated transaminase for the first time and received interferon therapy, but stopped antiviral therapy 6 months later due to poor efficacy. In July 2006, she suffered another attack and received interferon for four months. In June 2008, she was treated with a combination of interferon and ADV in the third episode. Interferon was discontinued after 10 months and ADV was taken continuously at 10mg/d. In February 2013, she developed pain in the bones and joints of both feet, which gradually spreads to the bilateral knees and hips and the whole body, eventually resulting in general weakness, ambulatory difficulty, reduced appetite and rapid wasting. She could only walk with the assistance of a cane and started to stay in bed all year round. Laboratory data revealed hypophosphatemia (0.45 mmol/L; normal range 0.80-1.60 mmol/L), hypouricemia (55 μmol/L; normal range 155-428 μmol/L) and hypocalcemia (2.13 mmol/L; normal range 2.25-2.75 mmol/L). In September 2014, laboratory test showed a reduced serum phosphate level of 0.57 mmol/L, serum uric acid of 61μmol/L (normal range 100-400μmol/L) and an increased ALP level of 219 U/L (normal range 35-129 U/L). She had normal parathyroid hormone (PTH, 21.46 pg/ml) and serum calcium (2.29 mmol/L). A urinalysis showed urine protein was elevated at 959 mg/L with urine occult blood 3+, suggesting impairment of renal tubular function. DXA measurement demonstrated osteoporosis in left femoral neck, femoral shaft, trochanter and total hip.

She had visited the department of stomatology, orthopedics, endocrinology, and gynaecology and obstetrics several times, and was diagnosed with osteoproliferation, perimenopausal anemia-associated climacteric syndrome. However, none of the treatments improved the symptoms. She stopped taking ADV since September 2014 and admitted to our hospital in February 2015.

On admission, she was 46.0 kg in weight and 154 cm in height and had a body mass index (BMI) of 19.4 kg/m^2. During the physical examination, she had ambulatory difficulty and generalized bone pain, especially in bilateral knees, lower extremity and ankles. Laboratory data revealed hypophosphatemia (0.64 mmol/L), hypohaemoglobin (95 g/L, normal range 115-150 g/L), elevated alanine aminotransferase (ALT, 113 U/L; normal range 7-40 U/L), aspartate aminotransferase (AST, 80 U/L; normal range 13-35 U/L) and erythrocyte sedimentation rate (ESR, 21 mm/h; normal range ≤ 20 mm/h). She was positive for hepatitis B surface antigen (HBsAg), hepatitis B e antibody (HBeAb) and hepatitis B core antibody (HbcAb), the level of HBV-DNA was 3.66×10^3 IU/ml. On the basis of her medication history, hypophosphatemia, proteinuria and lower BMD showed by DXA measurement, we made a diagnosis of hypophosphatemia osteomalacia and Fanconi's syndrome possibly secondary to ADV therapy.

Clinical pharmacist and infectivists cooperated together and developed personalized drug delivery protocol for this patient according to her condition. Firstly, she was HBsAg、HBeAb、HBcAb and HBV-DNA positive with elevated ALT and AST. According to *The guidelines of prevention and treatment for chronic hepatitis B*: for CHB patients with positive serum HBV DNA, if their ALT continues to be abnormal, antiviral therapy should be given[9]. ETV was commenced at a daily dose of 0.5 mg. Secondly, symptomatic hypophosphatemia or chronic phosphate wasting due to renal tubular defect is an indication for phosphate supplementation[10]. Parenteral phosphorus supplementation is generally reserved for patient with life-threatening hypophosphatemia and should not be used in patients with renal failure. With a serum

phosphorus level of 0.64mmol/L, positive urine occult blood, and elevated urine total protein, the safest mode of therapy for this patient is oral supplementation with Composite Potassium Hydrogen Phosphate (2ml/d). Besides, Calcitriol Soft Capsules (Rocaltrol®, 0.25mg, qd p.o.), Calcium Carbonate and Vitamin D3 Tablets (Caltrate®, 1.5g, bid p.o.) and Alendronate Sodium Tablets (Fosamax®, 70mg, qw p.o.) was used to normalize phosphate levels and decrease bone pain.

10 days after changing the treatment regimen, serum phosphorus levels returned to normal range thus phosphorus supplementation was discontinued. After 3 months, she reported that general bone pain and muscle weakness were ameliorated, her walking ability had improved significantly. Urine tests showed elevated β2-MG of 3.16 mg/L (normal range < 0.25 mg/L). One year after the discontinuation of ADV, she stopped taking calcium and alendronate. Urine tests after 19 months showed an increase in β2-MG to 1.18 mg/L (Figure.2). At the most recent follow-up, she was positive for HBsAg and HBeAb but negative for HBsAb and HBeAg, her liver function indicators and serum ion concentration are within the normal range. However, DXA measurements showed a continuously decreasing BMD in L1-4 and left femoral neck compared with the results of the 2014 examination as shown in Table 1. This suggests that ADV may cause long-term irreversible damage to bone health.

3. CONCLUSION:

ADV is a nucleotide analog that widely used in the treatment of HBV infection. It is excreted mainly by proximal renal tubule. The basolateral human organic anion transporter-1 (HOAT-1)[11] of the proximal renal tubule mediates its uptake from the blood into proximal tubular cells and multidrug resistance proteins (MRPs) 2, 4, and 5 are pertinent to ADV's secretion into the urine[12]. Polymorphism of the genes that encode these proteins, large doses or prolonged application of ADV will result in drug accumulation in renal tubular epithelial cells. ADV may inhibit mtDNA replication complex and rewire glucose metabolism[13]. The consequent renal tubular mitochondriopathy will impair the reabsorption function of the proximal tubular,

leading to increased urinary phosphorus excretion. The resulting hypophosphatemia stimulates bones to release phosphorus, which will cause defective bone mineralization, accumulation of non-mineralized bone-like tissue, thus leading to hypophosphatemic osteomalacia. Management of ADV-induced osteomalacia including changes in antiviral drugs and phosphate supplementation.

HO commonly manifest as fatigue, generalized muscle weakness, skeletal pain, and walking difficulty. Bone pain usually appears in the weight bearing sites, such as the bilateral lower limbs initially, and then spreads to the entire body. Due to the non-specific clinical manifestations and the non-routine serum phosphorus level test, ADV-induced HO is quite easy to be missed or misdiagnosed[7]. The misdiagnosis as osteoproliferation and perimenopausal anemia-associated climacteric syndrome prevented this patient from getting the appropriate treatment timely and brought severe pain to her life. It must be emphasized that both osteomalacia induced by ADV and induced by tumor are manifested as hypophosphatemia, high ALP, and increased bone turnover markers, but hypouricemia and proteinuria are specific to ADV-induced HO[6]. The patient's initial tests showed low serum uric acid levels, possibly due to malnutrition caused by a loss of appetite. Despite some perspective insisting that creatinine levels can be used to adjust the dose of ADV[14], this patient's creatinine levels were basically in the normal range, confirming the view that routine renal function tests based on serum creatinine are insufficiently sensitive to early renal structural and functional abnormalities[15]. Previous studies have identified fractional excretion of filtered phosphate (FEPO4)[10] and neutrophil gelatinase-associated lipocalin (NGAL)[16] as biomarkers in the early detection of renal tubular injury during long-term ADV treatment.

It seems that patients from East Asian are prone to developing nephrotoxicity and HO on low-dose ADV[6, 17]. Moreover, in a study that aimed to clarify clinical characteristics of ADV-related HO in Chinese patients with foreign CHB counterparts in which other Asians accounted for 84.4%, it was found that Chinese patients are more prone to develop HO earlier[15]. The reasons for this racial specificity might be

a result of high prevalence of HBV infection rate, ADV prescription preference, lower BMI, ABCC2 and hOAT-1 gene polymorphisms in Asian population.

It is generally believed that the incidence of ADV-induced nephrotoxicity is time- and dose-dependent and is reversible[18]. A recent study provided two cases of ADV-induced HO whose urine β2-MG persistently elevated after 22 and 54 months of the cessation of ADV respectively, raising concerns about the reversibility of ADV-related nephrotoxicity[7]. β2-MG a sensitive indicator of tubular function. In normal kidneys, after filtration through the glomeruli, about 99.9% of β2-MG is reabsorbed by the tubules, the remaining negligible amount is excreted from the urine. Elevated β2-MG levels in urine is an early marker of proximal renal tubular damage[19]. In the present case, the β2-MG level was continuously elevated after ADV's discontinuation for 8 to 19 months, indicating that kidney function did not recover for a long time. In previous studies, after 11-22 months of follow-up of patients with low-dose ADV induced HO, although there is an improvement of BMD on DXA, the T/Z-score remained low[17], underlining the importance of long-term follow-up of bone health after the cessation of ADV. In this patient, a six-year follow up revealed a deteriorating BMD in L1-4 and left femoral neck. Although chronic HBV infection and a perimenopausal period were risk factors of osteoporosis[20, 21], patients with ADV-induced HO usually get an increase in BMD after drug discontinuation[7, 17]. To the best of our knowledge, a sustained reduction in BMD after ADV withdrawal has yet been reported. The persistent elevated urine β2-MG and deteriorated BMD in our case may indicate that low-dose ADV-induced nephrotoxicity and osteotoxicity was not completely reversible in some patients.

In conclusion, we have presented the case of a 51-year-old Chinese woman with severe polyarthralgia, bone pain and muscular weakness from HO and Fanconi's syndrome caused by 6 years of ADV treatment. Laboratory profiling revealed severe hypophosphatemia, high ALP, and elevated urinary total protein. After the cessation of ADV and the commencement of ETV, as well as administration of treatment with alfacalcidol and phosphates, her clinical symptoms of Fanconi's syndrome improved

and CHB was better controlled, but the elevated β2-MG and decreased BMD persisted. To avoid the severe adverse reactions that occurred in this case, it is paramount for clinical pharmacists and hepatologist to prescribe appropriate dose of medication, monitor serum phosphorus levels, bone turnover markers, urinary ions as well as early indicators reflecting proximal renal tubular function regularly.

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Written informed consent was obtained from this patient.

CONFLICT OF INTEREST

Authors declared no conflicts of interest about the current publication.

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Author contribution

ZY: reviewed the literature and wrote this manuscript. LJF: diagnosed this adverse drug reaction and provided the treatment. TSM and WB: reviewed the literature and revised the manuscript. All authors: read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this case report are included in this published article.

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REFERENCES

1. Papatheodoridis, G., et al., *EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection.* Journal of Hepatology, 2012. **57**(1): p. 167-185.
2. Koumbi, L., *Current and future antiviral drug therapies of hepatitis B chronic infection.* World Journal of Hepatology, 2015. **7**(8): p. 1030-1040.
3. Gong, Z.J., et al., *Inhibitory effect of 9-(2-phosphonyl-methyoxyethyl) adenine (PMEA) on hepatitis B virus replication in vitro.* Journal of Hepatology, 1998. **28**(SUPPL. 1): p. 101-101.
4. Vigano, M., P. Lampertico, and M. Colombo, *Drug safety evaluation of adefovir in HBV infection.* Expert Opinion on Drug Safety, 2011. **10**(5): p. 809-818.
5. Law, S.t., K.K. Li, and Y.Y. Ho, *Nephrotoxicity, including acquired Fanconi's syndrome, caused by adefovir dipivoxil - is there a safe dose?* Journal of Clinical Pharmacy and Therapeutics, 2012. **37**(2): p. 128-131.

6. Wei, Z., et al., *Osteomalacia induced by long-term low-dose adefovir dipivoxil: Clinical characteristics and genetic predictors.* Bone, 2016. **93**: p. 97-103.
7. Qian, Y.-Y., et al., *Low-dose adefovir dipivoxil-induced hypophosphatemia osteomalacia in five chronic hepatitis B virus-infected patients. Is low-dose adefovir dipivoxil-induced nephrotoxicity completely reversible?* Drug Design Development and Therapy, 2019. **13**: p. 1127-1133.
8. Lv, Y.N., et al., *The clinical and pathological features of adefovir dipivoxil-related renal impairment.* Clinical Nephrology, 2019. **91**(3): p. 180-186.
9. Chinese Society of Infectious Diseases, C.M.A. and C.M.A. Chinese Society of Hepatology, *The guidelines of prevention and treatment for chronic hepatitis B (2019 version).* Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology, 2019. **27**(12): p. 938-961.
10. Assadi, F., *Hypophosphatemia An Evidence-based Problem-Solving Approach to Clinical Cases.* Iranian Journal of Kidney Diseases, 2010. **4**(3): p. 195-201.
11. Cihlar, T., et al., *The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1.* Molecular Pharmacology, 1999. **56**(3): p. 570-580.
12. Shimizu, M., et al., *Predictors of kidney tubular dysfunction induced by adefovir treatment for chronic hepatitis B.* World Journal of Gastroenterology, 2015. **21**(7): p. 2116-2123.
13. Zhao, X.B., et al., *Tenofovir and adefovir down-regulate mitochondrial chaperone TRAP1 and succinate dehydrogenase subunit B to metabolically reprogram glucose metabolism and induce nephrotoxicity.* Scientific Reports, 2017. **7**: p. 15.
14. Makita, T., et al., *Adefovir Dipivoxil-induced Fanconi's Syndrome and Osteomalacia Following Multiple Bone Fractures in a Patient with Chronic Hepatitis B.* Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan, 2019. **139**(4): p. 641-645.
15. Chen, N., et al., *Adefovir dipivoxil induced hypophosphatemic osteomalacia in chronic hepatitis B: a comparative study of Chinese and foreign case series.* Bmc Pharmacology & Toxicology, 2018. **19**(23).
16. Zhang, Y., et al., *Neutrophil gelatinase-associated lipocalin accurately predicts renal tubular injury in patients with chronic hepatitis B treated with nucleos(t)ide analogs.* Hepatology Research, 2018. **48**(2): p. 144-152.
17. Wu, C., et al., *Hypophosphatemic osteomalacia and renal Fanconi syndrome induced by low-dose adefovir dipivoxil: a case report and literature review suggesting ethnic predisposition.* Journal of Clinical Pharmacy and Therapeutics, 2013. **38**(4): p. 321-326.
18. Izzedine, H., et al., *Renal safety of adefovir dipivoxil in patients with chronic hepatitis B: Two double-blind, randomized, placebo-controlled studies.* Kidney International, 2004. **66**(3): p. 1153-1158.
19. Schardijn, G.H.C. and L.W.S. Vaneps, *BETA-2-MICROGLOBULIN - ITS SIGNIFICANCE IN THE EVALUATION OF RENAL-FUNCTION.* Kidney International, 1987. **32**(5): p. 635-641.
20. Huang, Z.F., et al., *Low bone mineral density in chronic Hepatitis B virus infection: A case-control study.* Pakistan Journal of Medical Sciences, 2017. **33**(2): p. 457-461.
21. Lo, J.C., S.A.M. Burnett-Bowie, and J.S. Finkelstein, *Bone and the Perimenopause.* Obstetrics and Gynecology Clinics of North America, 2011. **38**(3): p. 503-+.

Diagrams in this article:

Figure1.Timeline of medications.

Figure 2. Laboratory examination of the patient. A. Creatinine, B. Urea, C. Uric acid, D. Phosphorous, E. Alkaline phosphatase, F. β2-microglobulin. Red line: reference range.