

Bone mineral density and urinary hydroxyproline are already abnormal in newly diagnosed patients with epilepsy in this North Indian study

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Abstract

Background & Objective: Epilepsy may have an impact on bone health of the patients even before drug therapy is initiated, particularly in the developing countries. This is in view of long delay in diagnosis and lifestyle changes. Therefore, in this study, bone health markers like bone mineral density (BMD) and urinary hydroxyproline were assessed in newly diagnosed epilepsy patients. **Methods:** The BMD was assessed by DEXA scan, and 24 hour urine hydroxyproline was estimated colorimetrically in 25 newly diagnosed epilepsy patients. Other bone markers like calcium, phosphorus, vitamin D and alkaline phosphatase were also estimated. Results were compared with 25 age and sex matched healthy controls, and were analyzed statistically. **Results:** The BMD and vitamin D were found to be significantly decreased ($p < 0.05$) while serum alkaline phosphatase and urine calcium and phosphorus were observed to be significantly increased ($p < 0.05$) in epilepsy patients as compared to healthy controls. The difference in urinary hydroxyproline and serum calcium/ phosphorus in the two groups was not found to be statistically significant ($p > 0.05$).

Conclusions: Bone health is found to be already compromised in epilepsy patients in this study from North India. BMD and urinary hydroxyproline may act as simple, non-invasive, convenient and inexpensive markers to assess bone health in these patients.

INTRODUCTION

Epilepsy is a major public health problem worldwide affecting around 50 million people.¹ Fractures resulting from reduction in bone mineral density are an important cause of morbidity and mortality in these patients.² The costs of these fractures are high including loss of daily functioning and wages, visits to doctors/ hospitals, hospitalizations and visits to physiotherapists. The World Health Organization (WHO) has recognized this growing concern and developed an initiative to expand awareness and research in this area.³ Chronic therapy with antiepileptic drugs (AEDs) is known to lead to reduction in bone mineral density and abnormalities in calcium metabolism including hypocalcemia, hypophosphatemia, elevated levels of serum alkaline phosphatase and parathyroid hormone, reduced levels of biologically active vitamin D metabolites and is generally associated with rickets and osteomalacia. These effects have been mostly studied in patients on therapy with hepatic enzyme inducers like phenytoin, carbamazepine,

primidone and phenobarbitone, which are thought to induce enzymes leading to inactivation or breakdown of vitamin D. But hepatic enzyme inhibitors are also reported to lead to bone loss. Therefore, exact mechanism of adverse bone health seen with AEDs is still unclear.⁴

Epilepsy itself, in addition to AEDs, increases the risk of fracture by a variety of mechanisms. Fractures following falls are more common in epilepsy patients as compared to healthy controls. The majority of falls are not related to seizure activity as two thirds of falls are observed in the absence of seizures.⁷ Epilepsy is associated with a variety of neurological deficits leading to weakness, loss of co-ordination and impaired cognition and sensory modalities. These may be responsible for increased propensity of falls in these patients. Gait disturbances may also be associated with neurological side effects of AEDs.

The risk of poor bone health in epilepsy patients is 2-6 times greater than the general population.⁶ Bone health may be adversely affected in epilepsy

patients even before initiating therapy with AEDs. The life style in epilepsy patients may also be responsible for poor bone health. As some of these patients may lead a comparatively sedentary and indoor life style which may be out of concern of the patients and their family for provoking seizure or risk during seizures. The immobility, inactivity, less exposure to sun and fewer weight bearing activities are strong risk factors for poor bone health or osteoporosis. They are frequently associated with mild vitamin D deficiency.⁸ The actual duration of epilepsy is difficult to be ascertained in newly diagnosed patients of epilepsy in India. A significant proportion (approximately 22% urban and 90% rural) of population might not be receiving appropriate treatment for epilepsy due to factors like lack of access or knowledge of treatment facilities, poverty, cultural beliefs, stigma and poor health services infrastructure. This is also true for many other developing countries.⁵ All these may contribute to poor bone health even before commencing AED treatment.

Bone turnover markers may be conveniently used to estimate bone health. Commonly used bone formation markers include osteocalcin and bone specific alkaline phosphatase while bone resorption markers are collagen breakdown products in serum or urine.⁹ Hydroxyproline is a major component of protein collagen and contributes to its stability. The values of urinary hydroxyproline excretion reflect the bone remodelling by osteoclasts. Hence, it acts as an important marker of bone resorption.¹⁰ Bone mineral density (BMD) test is the primary test used to identify osteoporosis and low bone mass. One of the preferred and most accurate ways to measure BMD is dual energy X-ray absorptiometry (DEXA) scan. It uses a low energy X-ray to evaluate bone density in lumbar spine, hip and wrist areas. BMD is often reported in terms of peak bone mass in young adults. Both these markers can provide a fair insight into the bone health of the subject. Inadequate sun exposure and less physical activity in these patients might affect vitamin D production which is also a core factor in determining bone health.

The objective of this study was to assess bone health of newly diagnosed epilepsy patients before initiating AED therapy, based on BMD test and urine hydroxyproline levels.

METHODS

The present study was conducted on 25 newly

diagnosed patients of epilepsy and 25 age and sex matched healthy controls after obtaining informed consent and approval from institutional postgraduate board of studies. The diagnosis and classification of epilepsy was according to the International League Against Epilepsy classification 2010; with careful history, neurological examination, electroencephalography and neuroimaging (computerised tomography or magnetic resonance imaging).¹¹ The patients in the pediatric (<14 years) age group, with any history of organic bone abnormality, intake of drugs / supplements affecting calcium and vitamin D metabolism, pregnant or lactating females and other metabolic or endocrinal diseases affecting the bone health were excluded from the study.

All the patients and controls underwent DEXA scan using Hologic Explorer QDR series (S/N 90797, Hologic Inc, Waltham, USA) at L1-L4. Bone mineral density was measured and T-score (the difference in standard deviations between a given bone density and peak bone density in the normal reference population) and Z-score (age-adjusted T-score) were calculated.¹² Twenty four hours urine sample was collected for hydroxyproline, calcium, phosphorus and creatinine estimation while venous blood sample was analyzed for serum calcium, phosphorus and alkaline phosphatase. Hydroxyproline was estimated colorimetrically using modified Neuman and Logan method. In this method, hydroxyproline is treated with copper sulphate and hydrogen peroxide in an alkaline solution resulting in formation of pyrroline-4-carboxylic acid which upon acidification gets converted to pyrrole-2-carboxylic acid. It, then, condenses with p-dimethyl aminobenzaldehyde to produce a coloured complex whose optical density is measured at 540 nm.¹³ Serum alkaline phosphatase, urine creatinine and serum/urine calcium and phosphorus were estimated using Autoanalyzer (Rx Suzuka) and kits by Randox. Serum vitamin D levels were estimated using enzyme linked immunosorbent technique.¹⁴ Body mass index (BMI) was calculated for all the subjects using the formula $\text{weight} / \text{height}^2$ as Kg/m^2 .

The data was analyzed and compared using appropriate statistical analysis (student 't' test, ANOVA and Mann-Whitney U test, wherever applicable).

RESULTS

Of the 25 patients and controls, there were 13 females and 12 males in each group. The median

age for the patients was 26 years with a mean±SD of 26.5±6.9 years (15-45 years) while for the control group, median age was 25 years with a mean±SD of 25.6±6.5 years (15-47 years), with a p value of 0.3. BMI was also comparable in both the groups as shown in Table 1. The BMD and biochemical parameters are also shown in Table 1. Majority of the patients (53.4%) had partial seizures. No patient was suffering from any neurological or learning disability. The BMD was found to be significantly decreased (p=0.001) while serum alkaline phosphatase was observed to be significantly increased (p=0.005) in patients as compared to healthy controls. Vitamin D levels were found to be decreased in patients with epilepsy as compared to healthy controls (p=0.016). The difference in urinary hydroxyproline and serum calcium/ phosphorus in the two groups was not statistically significant (p>0.05).

DISCUSSION

The results of the present study point towards altered bone health in epilepsy patients on diagnosis before using AEDs. This is likely to be due to delay in the diagnosis of epilepsy. The delay in diagnosis may be due to lack of awareness and inadequate healthcare facilities in a developing country like India. Therefore, at diagnosis, the patient may already have long duration of epilepsy with lifestyle changes that affect the bone health.

Many studies have reported the development of osteopenia, osteoporosis and osteomalacia

in patients of epilepsy on AEDs. These drugs induce the catabolism of vitamin D producing the consequent effects.¹⁴⁻¹⁶ Not many studies are available to report on the bone health of epilepsy patients prior to start of AED therapy.¹⁴⁻¹⁶ The levels of serum calcium and phosphorus were not found to be significantly different from healthy controls (p>0.05) in this study, though levels of alkaline phosphatase were observed to be significantly increased in epilepsy patients as compared to healthy controls (p=0.005). Alkaline phosphatase is a marker of bone formation and indicates poor bone mineralization. Bone is a complex dynamic tissue and undergoes continuing remodelling process in discrete areas throughout the skeleton.¹⁷ Bone remodelling is intimately related to changes in expression of members of tumor necrosis factor (TNF) receptor superfamily on bone marrow stromal cells (e.g., the macrophage colony stimulating factor (M-CSF), the receptor activator of nuclear factor-kappa B ligands (RANKL), RANK and osteoprotegerin and various cytokines and calciotropic hormones). RANKL binds the RANK receptor on osteoclast precursors and induces the formation of osteoclasts by a special signalling pathway.¹⁸ Any disturbance in this system is bound to affect bone health. BMD was found to be significantly decreased in patients of epilepsy as compared to healthy controls (p<0.001). Though, the urinary levels of hydroxyproline were found to be increased in epilepsy patients as compared to healthy controls but the difference was statistically not significant (p=0.272). Hydroxyproline is a collagen breakdown product and the results

Table 1: Comparison of bone health parameters in epileptic patients and healthy controls

	Epileptic patients N=25	Healthy controls N=25	p Value
BMI (Kg/m ²)	22.64±3.4	23.4±3.2	0.09
BMD T-score	-1.25±1.10	-0.96±0.14	0.001
Z-score	-1.10±1.02	-0.90±0.16	0.001
Serum calcium (mg/dL)	10.10±1.11	10.95±0.74	0.160
Serum phosphorus (mg/dL)	4.38±1.10	3.74±0.74	0.475
Serum alkaline phosphatase (U/L)	121.56±45.83	83.03±12.83	0.005
Serum vitamin D (ng/mL)	32.93±6.38	38.04±7.95	0.016
Urine calcium (mg/day)	127.12±64.83	122.77±59.79	0.434
Urine phosphorus (mg/day)	696.69±221.09	692.36±249.76	0.295
Urine hydroxyproline (mg/day)	17.45±3.02	18.00±2.55	0.272

suggest that the process of collagen breakdown has not started in these patients. But the bone mineral composition is already compromised as is seen by decreased BMD. Urinary calcium and phosphorus levels were also found to be increased as compared to healthy controls but the difference was not significant statistically. These findings may be explained on the basis of vitamin D insufficiency in these patients. Vitamin D deficiency is quite prevalent in Indian population and patients suffering from epilepsy are more prone to it because of a relatively sedentary and indoor lifestyle once they suffer from the disease.¹⁹ In this study vitamin D levels were found to be within the normal range (> 30 ng/mL) though levels were found to be significantly decreased ($p=0.016$) in patients as compared to controls.

BMD represents a coordinated action or the balance between bone resorption or the bone depleting action of osteoclasts and the bone-formative action of osteoblasts (bone remodelling). Osteoclast and osteoblast dynamics, body weight, exercise, vitamin D, calcium, and phosphorous homeostasis, lack of active metabolite of vitamin D (1,25(OH)₂D) as well as connective tissue arrangements can alter bone structure and architecture.^{20,21} Both these markers, i.e. DEXA scan for BMD and urine hydroxyproline are non-invasive, inexpensive and can be repeated without hassles and reflect metabolic activity and mineralization of the skeletal system. These can be effective tools for screening in epileptic patients.^{20,21}

The limitations of our study include small sample size, not estimating parathyroid hormone and including DEXA scan of lumber area only.

Even with the many limitations, this study points towards a compromised state of bones in epilepsy patients. BMD appears to be a sensitive marker and it may be used to detect defective mineralization at an early stage. The process of collagen breakdown cannot be commented upon as urine hydroxyproline levels were not found to differ significantly from controls but it may prove to be a useful marker to detect damage by AEDs.

Thus, it can be concluded that bone health may be already compromised in epilepsy patients at diagnosis in India, and it may be worsened further by treatment. The findings call for a cautious selection of AEDs, as the newer drugs may be less deleterious to bone health, though this is yet to be proven. The patients may also be instructed for minimizing alcohol/smoking, carrying out weight bearing exercises, introducing healthy balanced

diet and ensuring sufficient vitamin D intake, which are factors that also affect bone health.²² BMD and urinary hydroxyproline may be used as effective screening biomarkers, especially BMD, but further studies with larger sample selection is required to confirm our findings.

DISCLOSURE

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