MANAGEMENT OF PHARMACOLOGICAL COMPLICATIONS IN ANTI-EPILEPTIC DRUG INDUCED DEGENERATIVE DISEASE

Thesis

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By

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Supervisor's Certificate

This is to certify that Ms. Sagarika Kabra has completed the necessary academic turn and the swirl presented by her is a faithful record is a bonafide original work under my guidance and supervision. She has worked on the topic "Management of Pharmacological Complications in Anti-Epileptic Drug Induced Degenerative Disease" under the School of Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow.

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Declaration by the Scholar

I hereby declare that the work presented in this thesis entitled "Management of Pharmacological Complications in Anti-Epileptic Drug Induced Degenerative Disease" in fulfilment of the requirements for the award of Degree of Doctor of Philosophy, submitted in the Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow is an authentic record of my own research work carried out under the supervision of Dr. A.K.S Rawat. I also declare that the work embodied in the present thesis-

i) is my original work and has not been copied from any journal/ thesis/ book; and
ii) has not been submitted by me for any other Degree or Diploma of any University/ Institution.

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ABSTRACT

The disease Epilepsy is known to be a one of the most common neurological disorders that affects people of almost all ages, races, social classes and different geographical locations. This disease of brain is characterized by more predisposition to generate seizures and by the neurobiologic, cognitive and other consequences of seizure remanence. The International League Against Epilepsy (ILAE) defines epilepsy as any of the following: (1) at least two unprovoked (or reflex) seizures that happen more than 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of additional seizures that happen within the next 10 years that is comparable to the general recurrence risk (at least 60%) following two unprovoked seizures; and (3) diagnosis of an epilepsy syndrome.

Regulatory bodies have approved a number of antiepileptic drugs (AEDs), including acetazolamide, carbamazepine, clonazepam, clorazepate, ethosuximide, ethotoin, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, mephenytoin, methsuximide, oxcarbazepine, phenobarbital, phenytoin, pregabalin, primidone, tiagabine, topiramate, trimethadione, valproate, vigabatrin, and zonisamide.

The acute treatment of status epilepticus mostly involves the administration of the following additional agents: diazepam, fosphenytoin, lorazepam, midazolam, and propofol. Practically speaking, the patient profile—which includes the drug's effectiveness for the seizure or epileptic syndrome, tolerability, safety, convenience of use, pharmacokinetics, and cost—must be taken into consideration when selecting an AED among first-line medications. Most epileptic patients can effectively manage their seizures with the use of an AED. Three-quarters of individuals with uncontrolled epilepsy have seizures, 5% recur, and about 65% of patients with new-onset epilepsy react. Changes in lifestyle can prevent seizure precipitation, especially in adolescents.

The diagnosis of epilepsy and the epileptic syndrome should be reevaluated if two or three medication regimens have not resulted in complete seizure control. If refractory epilepsy is confirmed, surgical alternatives should be considered in suitable individuals. An ideal AED can be simply administered once or twice a day, is metabolically inert, does not cause adverse drug interactions, and does not require monitoring of plasma concentrations. Regrettably, some routinely used traditional AEDs, including carbamazepine (CBZ), phenobarbital (PHB), and phenytoin (PHT), stimulate or, less frequently, inhibit the cytochrome p450 system, or inhibit glucuronidation-related enzymes, such valproate (VPA).

Thankfully, there are now AEDs that are less likely to cause enzyme induction-based drug interactions, like oxcarbazepine, or that are not metabolized by the oxidative cytochrome p450 system at all, like gabapentin (GBP), levetiracetam (LEV), lacosamide (LCM), lamotrigine (LTG), pregabalin (PGN), topiramate (TPM), and zonisamide (ZNS). AED-related bone loss is typically subtle and asymptomatic at first, going unnoticed for a long time and frequently untreated. The use of AEDs has been linked to a number of biochemical abnormalities, including hypophosphatemia, hypocalcaemia, decreased serum levels of vitamin D (biologically active metabolite), and elevated parathormone (PTH) levels.

Changes in bone metabolism have been linked in preclinical trials to oxcarbazepine, gabapentin, and levetiracetam. One typical explanation for bone loss in epileptic patients is a deficiency in

vitamin D, which is necessary for bone remodelling and growth. It has been discovered that stimulating the hepatic CYP450 system speeds up the breakdown of vitamin D into its polar inactive metabolites, reducing the amount of vitamin D that is physiologically active.

Being an enzyme inhibitor, valproic acid is also linked to a drop in bone mineral density and an elevated risk of fractures. Consequently, it has been proposed that AEDs may impact bone metabolism by pathways other than hepatic enzyme activation.

Low bone mass, deteriorating bone tissue, and alteration of the microarchitecture of the bone are the hallmarks of osteoporosis, a disease that can impair bone strength and raise the risk of fractures. The most prevalent bone disease in humans, osteoporosis is a serious public health issue. It is more prevalent in older adults, women, and Caucasians. Just as hypertension increases the chance of stroke, osteoporosis does the same for fractures. Many people of all ages and genders suffer from osteoporosis, and as the population ages, so will the prevalence of the condition. It is a disorder that remains undetected until fractures happen, which can lead to serious secondary health issues or even death. The most commonly prescribed medications for osteoporosis treatment are bisphosphonates. Estrogen has never been licensed for the treatment of osteoporosis; nonetheless, estrogen replacement therapy (ERT) is used to prevent postmenopausal osteoporosis in patients who have a considerable risk of osteoporosis and for whom non-estrogen medicines are deemed inappropriate.

Dual energy x-ray absorptiometry scan (DEXA) readings are used in clinical practice to diagnose osteoporosis. The World Health Organization states that osteoporosis is diagnosed when bone mineral density is 2.5 standard deviations below the mean for normal Caucasian women. However, it is frequently maintained that this definition overemphasizes bone quantity at the expense of bone strength. It has been suggested that osteoporosis diagnosis cannot be achieved in the absence of a clinically diagnosed fracture. It has been demonstrated that fracture risk is independent of bone mineral density and is correlated with a loss of connection within the trabecular bone network. Therefore, in an effort to gain a deeper understanding of bone microarchitecture, various diagnostic methods have been employed recently.

Raloxifene is a selective modulator of estrogen receptors that exhibits estrogen antagonistic effects in endometrial and breast tissue while also partially mimicking the effects of estrogen in bone and the cardiovascular system.

When raloxifene 30 to 150 mg/day was administered instead of a placebo, postmenopausal women and patients with osteoporosis consistently and significantly lowered their serum and urine markers of bone turnover. In a 6-month research, the effects of conjugated equine estrogen (0.625 mg/day) on bone turnover were greater than those of raloxifene 60 mg/day. Over the course of a year, raloxifene 60 mg/day + alendronate 10 mg/day seemed to have a higher impact on indicators of bone turnover than either medication alone.

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Abbreviations	Description
ILAE	International League Against Epilepsy
AED	Anti- Epileptic Drugs
PHT	Phenytoin
CBZ	Carbamazepine
PRM	Primidone
PB	Phenobarbital
BMD	Bone Mineral Density
ATP	Adenosine triphosphate
SERMs	Selective estrogen receptor modulators
SVP	Sodium Valproate
ECM	Extracellular matrix
CTX-I	Type -I collagen
TALP	Total Alkaline Phosphatase
DAN	Differential Screening chosen gene aberrative in neuroblastoma
DKK 1	Dickkopf-1
DXA	Dual energy X-ray absorptiometry
PMW	Post-menopausal women
RLX	Raloxifene
CVD	Calcium Vitamin D
IAEC	Institutional Animal Ethics Committee

LTM	Levetiracetam
HxP	Hydroxyproline
TRAP	Tartrate Resistant Acid Phosphatase
MES	Maximal Electroshock Induced Seizures
HRP	Horseradish Peroxidase
BMC	Bone mineral concentration
TGF-3	Transforming Growth Factor -3
DEXA	Dual energy X-ray absorptiometry
OPG	Osteoprotegerin
HLE	Hind Limb extension

CHAPTER 1
INTRODUCTION

1.1 INTRODUCTION

1.1.1 EPILEPSY

The disease epilepsy is known to be a common disease affecting the nerves of the brain and areas related to it. The word "epilepsy" has been used since 500 BC. It means "attack" or "assault" in Greek.

The International League Against Epilepsy (ILAE) commission report by Fisher et al. (2005) says that it is a long-term condition marked by repeated, unprovoked epileptic seizures. An epileptic seizure is a short-lived set of various symptoms caused due to improperly high activity of brain cells. Epilepsy usually has effects on the brain, mind, emotions, and social life. It affects people of all ages, but it is most common in the first year of life and in people over 65(Hauser et al., 1993).

Up to 1% of people have epilepsy, making it the second most frequent major neurologic illness after stroke. Ninety percent of the 50 million or so epileptics worldwide reside in underdeveloped nations. Over the last few years, there have been several advancements in our understanding of epilepsy. It is a common chronic neurological condition marked by recurrent unprovoked seizures and an unbalanced balance between cerebral excitability and inhibition toward excessive excitability.

Epileptic seizures frequently result in a momentary loss of consciousness, putting the sufferer at danger for physical injury and frequently making it difficult for them to pursue their career or schooling. It transcends all borders, including those related to age, gender, geography, socioeconomic class, and race. Although it can happen at any age, epilepsy is more common in young children and adults over 65. Although epilepsy is a syndrome characterized by a wide range of symptoms involving aberrant electrical activity in the brain on an episodic basis, it is not a single disorder. Not every epilepsy syndrome lasts a lifetime; some only occur during specific childhood developmental phases.

1.1.2 HISTORY OF EPILEPSY

The Greek word "epilepsia," which means "to take hold of," is where the word epilepsy originates. Epi, which means "upon," and lambanein, which means "to take," are joined to produce this word. Epilepsy in antiquity was thought to be a sign of a demonic possession or fainting spell. In the past, epilepsy was regarded as a sacred illness. Many people held this belief by believing that epilepsy affected those who had been partially captured by demons or that the gods had sent epileptics to have visions.

It came to notice that the ancient Egyptians also have epilepsy which is evidenced by Edwin Smith Surgical Papyrus that dates to about 1700 BC. Its further details for multiple epilepsy accounts in which one is particularly of intriguing. On the basis of Egyptian records on the basis of an instance in which a physiological reaction that was triggered by direct brain stimulation. Aman with " a gaping wound in his head" as described in the case as "shuddering exceedingly" as the wound was palpated. Then the Egyptians showed that the seizures might be brought on by disruption of the cortical layer that sets it apart from the Mesopotamians that thought seizures were caused by spirits & gods. Then an upcoming source about epilepsy is also found in Chinese writings from 770-221 B.C.

The pathophysiology of epilepsy, which is rooted in spirituality, was not thoroughly questioned until the 5th century BC, when the Greek School of Hippocrates postulated that epilepsy could have its origins in the brain. Hippocrates thought that epilepsy, which was called "sacred" because of its unusual and mysterious appearance, was not any more divine than other illnesses. In addition, he proposed the theory that, unlike other illnesses, epilepsy might be healed before it develops chronic and becomes incurable.

Specifically, Hippocrates was the first in proposing that epilepsy is inherited rather than communicative and which connect it to the brain. As per these thoughts, the condition assumes that clinically as unilateral motor indications connected by an aura. This further acts as an warning indicator the enables the patient to fast withdraw it from the people to avoid convulsions. The present belief at the time is that spirits were the main source of epilepsy that contributes to the shame that is associated with its conditions in the society. He said that the dread that society had developed around epilepsy was the reason for its misperception and response to the condition. Hippocrates was among the first to offer a secular explanation for epilepsy, but regrettably, his theory had little impact on the belief in the paranormal for many centuries to come.

In Europe, a Hippocrates theory that says that epilepsy was a brain disorder that began to take place in the 17th century and further continued into the millennium. Traité de l'épilepsie, which is written by a well-known Swiss Physician Samuel Tissot (1728-1797) that was published in 1770. Then a four-volume work title Traite des Nerfs et du leurs Maladies that he published after ten years that solidify his reputation as a leading physician of the Era. Scottish physician William Cullen (1710-1790) described as how seizures could happen at any place in the body and not always it is linked with unconsciousness. Maisonneuve (1745-1826) who is a French physician, stressed the importance of hospitalizing the patients of epilepsy at that period of time.

Three cells make up the majority of the cellular component of bone: osteoblasts, osteoclasts, and osteocytes. Osteocytes play a mechano-sensory role in the development of bones and are located in the matrix's lacunae. While osteoclasts resorb bone enzymatically, osteoblasts produce osteoid. For bone formation and remodeling over the life course, all three subtypes are crucial.

1.1.3 EPIDEMIOLOGY

One of the most prevalent severe neurological conditions is epilepsy. An estimated 55 individuals with 17 cases of epilepsy are thought to reside in India, 20 in the USA, and 3 in the UK. In the United States, 120 out of every 100,000 individuals seek medical attention annually due to a newly identified seizure. In people without epilepsy, at least 8% will experience at least one seizure. Within 5 years, 23% to 80% of people who had their first unprovoked seizure would experience another one. Six Epilepsy occurs in 44 out of every 100,000 people annually, age-adjusted.

Approximately 125,000 new cases of epilepsy are diagnosed each year, with 30% of those cases involving individuals under the age of 18. It is widely acknowledged that epilepsy affects the elderly very frequently. In long-term care facilities, antiepileptic drugs (AEDs) are taken by at least 10% of patients. This significant issue was brought to light by the Epilepsy Bereaved National Sentinel Audit of Epilepsy-Related Deaths. According to the audit, "1,000 deaths resulting from epilepsy occur every year in the U.K." The majority of these deaths are linked to seizures, accounting for 42% of possibly preventable deaths.

1.1.4 CAUSES OF EPILEPSY

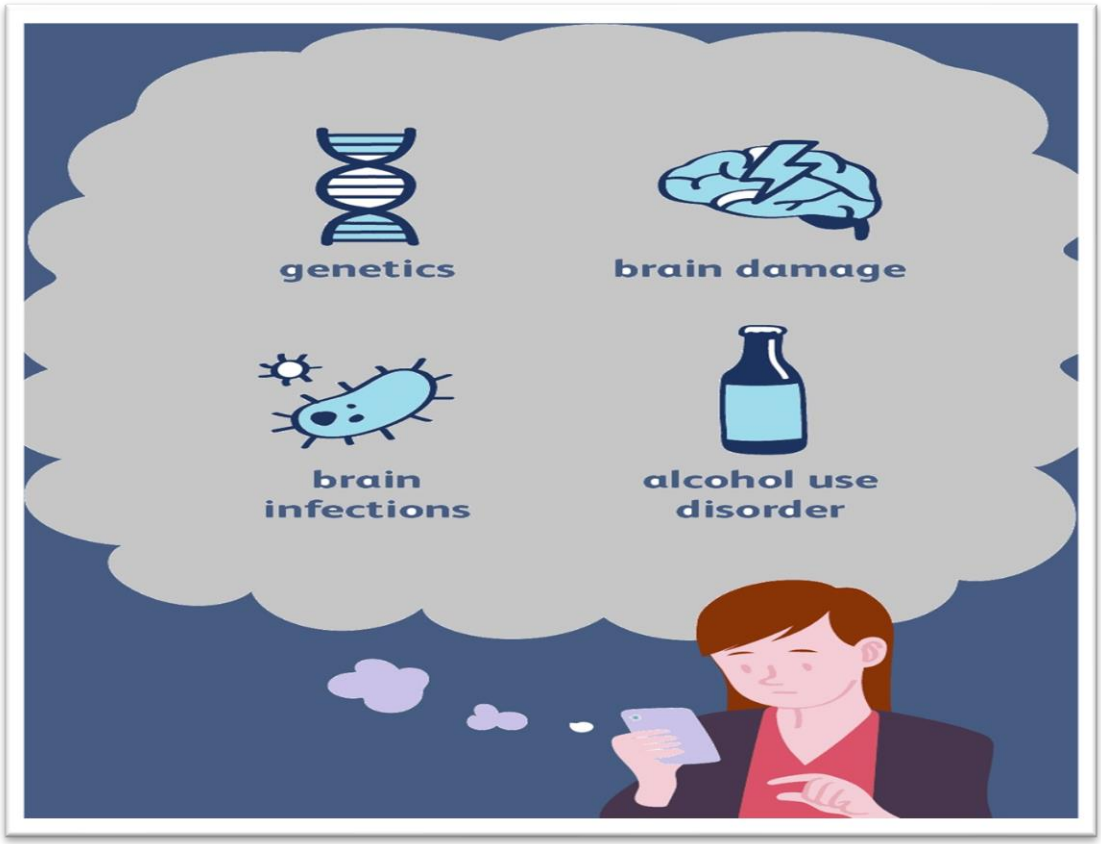

Fig. 1.1: Causes of Epilepsy

We don't know the cause of epilepsy at all. The term "epilepsy" does not describe the kind or severity of a person's seizures; epilepsy can be brought on by genetics in certain situations, but it can also be brought on by strokes, brain tumors, infections, high fevers, or blows to the head. Although it can affect persons of any age, it has been shown that inheritance, or genetics, plays a significant part in many causes of epilepsy in very young children. For example, not all people with a major head injury—a known cause of seizures—will go on to have epilepsy.

Patients with epilepsy often cite specific triggers or precipitants for their seizures, such as reading, flashing lights, emotional stress, sleep deprivation, heat stress, alcohol, and feverish illness. These precipitants are known as reflex epilepsy syndromes.

There are many age groups the experience epilepsy having different reasons. As in newborns & early infancy stages, CNS infections, CNS abnormalities (congenital), metabolic disorders are the commonly known causes of epilepsy. The cause that is more common of febrile seizures in late infancy & early childhood is due to CNS infections & trauma. The syndromes that are well defined are mainly seen in children. In that case the causes are mainly secondary to any CNS damage in adolescence & adulthood. The cause that is more common is of cerebro-vascular diseases in the elderly which is brain trauma, CNS malignancies and various others degenerative illness.

1.1.5 PATHOPHYSIOLOGY: EPILEPSY

Paroxysmal expressions of the cerebral cortex are seizures. An abrupt imbalance between the excitatory and inhibitory forces within the network of cortical neurons causes a seizure. The fundamental physiology of a seizure episode is found in an unstable cell membrane or the supporting cells that surround or are next to it. Any cortical or subcortical region's gray matter is the source of the seizure. A tiny percentage of neurons fire improperly at first. A focal seizure can occur locally due to normal membrane conductance, inhibitory synaptic current breakdown, and excess excitability, or more broadly due to generalized seizure. Through physiological processes, this onset spreads to nearby remote places.

The seizures could be due to abnormality in conductance of potassium, voltage activated ion channels malfunctioning, deficiency in the membrane ATPase that is involved in ion transportation process. GABA & dopamine inhibits the activity of neurons with its propagation with that certain neurotransmitter such as glutamate, aspartate, acetyl-choline, histamine, peptides etc. increases the excitability of neurons with its propagation.

The need for blood flow to the brain increases during a seizure in order to remove carbon dioxide and provide substrate for the neurons' metabolic activity. The longer the seizure lasts, the more ischemia the brain experiences, which increases the risk of neuronal death and other brain damage.

1.1.6 MANAGEMENT OF EPILEPSY

There is interchangeability between the terms anticonvulsant and antiepileptic. An antiepileptic medication is one that is used medicinally to regulate epilepsies, and an anticonvulsant is a substance that prevents seizures in laboratory animals caused by experiments.

PRINCIPLES OF EPILESPY MANAGEMENT

There are different principles for managing epilepsy as;

- If there is any causative factor of epilepsy than it must be treated properly such as cerebral neoplasm.
- The patients should have good education of the disease, treatment duration & compliance.
- Certain precipitating factors should be removed or avoided such as alcohol, emotional stress and others.
- The natural variations should be anticipated as fits might occur mainly or exclusively at the time of periods in women.
- The anti-epileptic drugs should be given at the time only when seizure and frequency is required that is more than one fit every 6 to 12 months.

1.1.7 OCCURRENCE OF EPILEPSY

There are no socio-demographic boundaries of this disease and an estimation of 50 million people globally are affected. The disease epilepsy is the most common neurological disorder with point prevalence of 4 to 10 per 1000 people as per the studies conducted previously. The rate of incidence is estimated around 50-60 per 100,000 persons-years and upto 8% of those people having at least one seizure in the complete lifetime (Fiest KM et al., 2017).

1.1.8 CLASSIFICATION

There is an international classification of epilepsy as well as epileptic seizures that was taken in 1969 by the International League against Epilepsy that was published in the year 1970 in Epilepsia (Gastaut, 1970).

As the seizure has been classified and the person is found to have epilepsy then it is classified as;

There are different types of epilepsy as focal, generalized, combination of focal & generalized and the last is unknown type of epilepsy. To see what the person has, all seizures type of patients should be defined and the data must be combined to see what kind of epilepsy.

When a patient is diagnosed with focal epilepsy as they experience focal conscious cognitive into bilateral tonic clonic seizures that comes from the left & right temporal lobes. There is combination of focal & generalized epilepsy if they feel both the types of seizures.

The cause should be ascertained as when the type of epilepsy has been identified. The classification of epilepsy is defined on the basis of following etiology as structural, genetic, infectious, metabolic, immunological and some are unknown. A patient could have more than one etiology and which is not hierarchical (Scheffer et al., 2017).

When a particular disease-causing mutation in a gene or copy number causes epilepsy, the condition is categorized as having a genetic etiology. Notably, if a patient's family history, electroencephalogram, and seizure semiology support the diagnosis of genetic epilepsy, genetic testing is not required. As patients who have focal aware auditory to bilateral tonic-clonic seizures along with EEG findings of right lateral temporal seizures as well as multiple family members who also shows the same semiology can be checked that is based upon the presentation with its family autosomal dominant lateral temporal lobe epilepsy (Vezzani A et al., 2016).

1.1.9 EPILEPSY IN WOMEN

Approximately 1.5 million women in India who are of reproductive age suffer from epilepsy (WWE), making up one-sixth of all WWE worldwide. Of them, 52% fall within the reproductive (15–49-year-old) age range (Thomas SV, 2011).

Catamenial epilepsy is the term used to describe the increased risk of seizures during menstruation that over 30% of WWE athletes report. According to a study, the ovulatory phase carried a higher risk of seizures than the anovulatory phase (Thomas SV et al., 2013).

1.1.9.1 CATAMENIAL EPILEPSY

The definition of catamanial epilepsy is a two-fold increase in seizure frequency during or shortly before menstruation. The incidence of catamenial epilepsy varies from 10% to 78%, depending on the diagnostic and data collection method employed. Three forms of catamenial epilepsy were described by Herzog and colleagues: luteal, periovulatory, and perimenstrual. They also described the seizure patterns of 184 females who had partial seizures of refractory complex in relation to their ovulatory cycle.

Seizures may occur more frequently if progesterone or estrogen levels are raised. More precisely, it has been discovered that progesterone or its metabolites (allopregnanolone) reduce neuronal excitability via influencing GABA A receptors. It is thought that estrogen stimulates the NMDA receptor in the hippocampus, which in turn increases seizure activity. An ovulatory female's greatest risk for catamenial seizures occurs right before ovulation and right before menstruation,

during which time her estrogen/progesterone ratio is highest. Catamenial seizures in anovulatory females usually happen in the second half of the menstrual cycle as a result of unopposed increased estrogen levels.

1.1.10 HOW EPILEPSY IS TREATED

During this time, the International League Against Epilepsy (ILAE) also expanded in size and prominence. It also started to have an impact on epilepsy treatment, primarily through the creation of "commissions," the most significant of which was the Commission on Antiepileptic Drugs, and the active participation of its leaders in drug therapy. Information about these novel medications was showcased at ILAE conferences, and Epilepsia was becoming the go-to publication for publishing in this field. The ILAE centenary history goes into great length about these topics. (Shorvon et al., 2009b).

There are a number of AEDs that causes voltage & frequency dependent decrease in the conductance by attaching to the sodium channel in its inactivated state. This category includes PHT, CBZ, LTG, oxcarbazepine, eslicarbazepine. Lacosamide that mainly affects slow inactivation of the sodium-channel but all the other sodium blockers modulate its fast inactivation (Beydoun et al., 2009).

The goal of treating epilepsy is to stop seizures for good without causing any other problems. So, the first AED should be chosen based on how well it works for the type of seizure or epilepsy syndrome the patient has and how well it works for the patient, preferably using data from well-designed randomized controlled trials.

The drug Sodium Valproate is generally used in treating primary generalized epilepsies. In the case of controlled trial, valproate has proven to be effective in controlling the seizures at 80 to 90% as carbamazepine or phenytoin for treating primary generalized tonic-clonic convulsions (Shakir et al., 1981).

The use of Valproic acid is effective in case of partial & generalized seizures as it is indicated as a monotherapy & adjunctive therapy for complex partial seizures that starts with a very small area of brain. These seizures might occur either in isolation or in association with the other types of seizures (Lowenstein DH et al., 2008).

For epilepsy with a recent onset, both newer and older AEDs are typically equally effective. Newer medications typically have fewer side effects. The American Academy of Neurology's guidelines were created to offer a framework for making decisions regarding in-patient care. They were not meant to preclude the use of any other legitimate alternative treatments, but rather were developed after a thorough analysis of the available clinical data. Therefore, a typical AED (carbamazepine, phenytoin, valproic acid/divalproex, phenobarbital) or a more recent medication (gabapentin,

lamotrigine, oxcarbazepine, topiramate) can be used to treat patients with recently diagnosed epilepsy. The decision is based on the unique features of each patient. Lamotrigine is one of the possibilities for kids whose absence seizures have just been diagnosed.

AEDs may be chosen based on factors other than how well they work and how well they work. Randomized controlled trials may not be able to capture these factors. Some of these are rare idiosyncratic reactions, effects that can cause birth defects, and long-term side effects. Effects on enzymes and the possibility of drug interactions are also important, as are the availability of parenteral formulations and the fact that in some cases, a target dose can be reached quickly. In fact, all of the existing guidelines stress how important it is to think about how each patient is different when choosing an AED. In addition to the type of seizure, factors like childbearing potential, old age, and comorbidities are also important.

The predicted effectiveness of AEDs has to be weighed against their possible side effects, and the risks of not getting treatment have to be taken into account as well. These risks should be looked at from the patient's point of view, taking into account not only the risk of more seizures but also the risk of morbidity and death caused by seizures, as well as the risk of AED toxicity. In an ideal world, figuring out the possible benefits of treatment would require knowing how epilepsy progresses on its own and what happens when it does (Perucca and Tomson, 2011).

Lastly, the choice of AED therapy is also affected by the cost of medications, how they are paid for, and the specific pros and cons of each drug. In the table 4 below, we talk about the pros and cons of each type of AED.

1.2 BONE HEALTH AND ANTI-EPILEPTIC DRUGS

Epilepsy is a common neurological condition that often needs to be treated with antiepileptic drugs (AEDs) for the rest of your life. Prior research has shown that people who use AEDs for long periods have less bone mass and are more likely to break bones (Kruse, 1968).

AEDs which stimulate the cytochrome P450 enzyme system, such as phenytoin (PHT), carbamazepine (CBZ), primidone (PRM), and phenobarbital (PB), are most frequently linked to altered bone metabolism and decreased bone density (Seth RD et al., 1995).

Bone is a living, breathing tissue that undergoes constant remodeling. Osteocytes track the mechanical stresses on the bone, osteoblasts are specialized cells that start the production of new bone, and osteoclasts break down existing bone. The dynamic equilibrium between bone production and bone resorption determines bone density. Osteoblasts deposit an organic matrix at the beginning of formation, which is followed by the mineralization process. Ninety to ninety-five percent of the organic matrix is made up of type I collagen, with additions from additional proteins like as thrombospondin, osteocalcin, osteonectin, and osteopontin. (Holick MF, Krane SM, 2001).

It is possible to assess a variety of biochemical markers that indicate the general rate of bone remodeling. These can be separated into indicators of bone resorption, which represent the broken-

down byproducts of osteoclastic activity, and markers of bone creation, which come from osteoblasts.

The disease epilepsy increases the chances of fractures through many kinds of mechanisms with increased use of AEDs. The fracture rate in patients with epilepsy is 2–6 times higher than the rate observed in the general population (Espallargues H et. al., 2001).

The fractures that are related with osteoporosis are most commonly seen in men & women that have came to an age of menopause. The industrialized nations that have a very high prevalence of osteoporosis having an average probability of sustaining an osteoporosis induced fracture in its complete course of life is about 2030 % in men & around 4056 % in women at the age of 50 (Lippuner et al., 2009). The disorder osteoporosis is a kind of skeletal disorder that is known to be compromised by bone strength, high vulnerability in increased chances in developing bone frangibility & fractures.

The integration of bone density and bone quality is the primary indicator of bone strength. The quantity of bone loss and peak bone mass are the factors that affect bone density. Architecture, turnover, damage accumulation (such as microfractures), and mineralization are all considered aspects of bone quality. An osteoporotic bone fractures when even a small force is applied to it. Osteoporosis is therefore a major fracture risk factor.

Vitamin D exhibits its effects at different levels in the process of bone mineral homeostasis. Apart from helping in maintaining the serum level of calcium & phosphorus with its physiological range by its work in the intestinal area and kidney. Along with it also regulates the differentiation with its functions of osteoclasts & as well as osteoblasts (Brown et al., 1999).

For research into how AEDs affect bone and how anti-osteoporotic regimens work in epilepsy, suitable animals are needed to study these things in the right way. Even though most previous studies looked at the effects of AEDs on bone in lab rats, there are animal models of osteoporosis in mice that use drugs like glucocorticoids or surgery to remove the ovaries. After three months of treatment, we have made an animal model of bone loss in male mice with 35 mg/kg PO-phenytoin (PHT). In mice, very few studies have looked at the effects of sodium valproate (SVP) and LTM on bone mineral density (BMD) and other indicators of bone health.

The presented research becomes more unique as we wondered that would these drugs cause osteoporosis. As most of the studies showed these effects of anti-epileptic medicines on bones as they have used male animals but we decided to use female mice in our study to see if these anti-epileptic drugs have different effects on bone as based on sex.

1.3 BONE HOMEOSTASIS

In order to preserve skeletal structure, serum calcium and phosphate homeostasis, and the ability to heal micro-damage, bone remodeling requires a precise balance between the actions of osteoclasts and osteoblasts. Along the surface of the bone, clusters of these cells within multicellular units form active remodelling sites, each of which is covered in a cell canopy. It has

been discovered that the mesenchymal stem cells (MSC) that surround the red bone marrow are the source of this cell canopy, which serves as a source of progenitor cells during the remodelling process [9]. Five successive stages of a remodelling cycle—activation, resorption, reversal, creation, and termination—occur on the surface of resting bones.

1.3.1 ACTIVATION

Osteocytes that express RANKL, or receptor activator of nuclear factor (NF) Kappa-B ligand, interact with the RANK receptor on osteoclast precursors to potently induce differentiation into multinucleated osteoclasts, thereby activating the resting bone surface. Additionally, osteoblasts that express macrophage colony-stimulating factor (M-CSF) increase the survival and development of osteoclast precursors. In order to attract osteoclast precursors, osteoblasts release chemokines. Additionally, they secrete matrix metalloproteinases, which break down unmineralized osteoid and reveal adhesion sites where osteoclasts can adhere.

1.3.2 RESORPTION

Hydrogen ions and lysosomal enzymes, such as cathepsin-K, are secreted by osteoclasts into a "sealed zone" that lies beneath the cell. They eliminate an old bone tunnel via proteolysis and acidity. By preventing osteoclast differentiation and accelerating their apoptosis, osteoprotegerin (OPG) can limit the RANK-RANKL connection and hence reduce resorption.

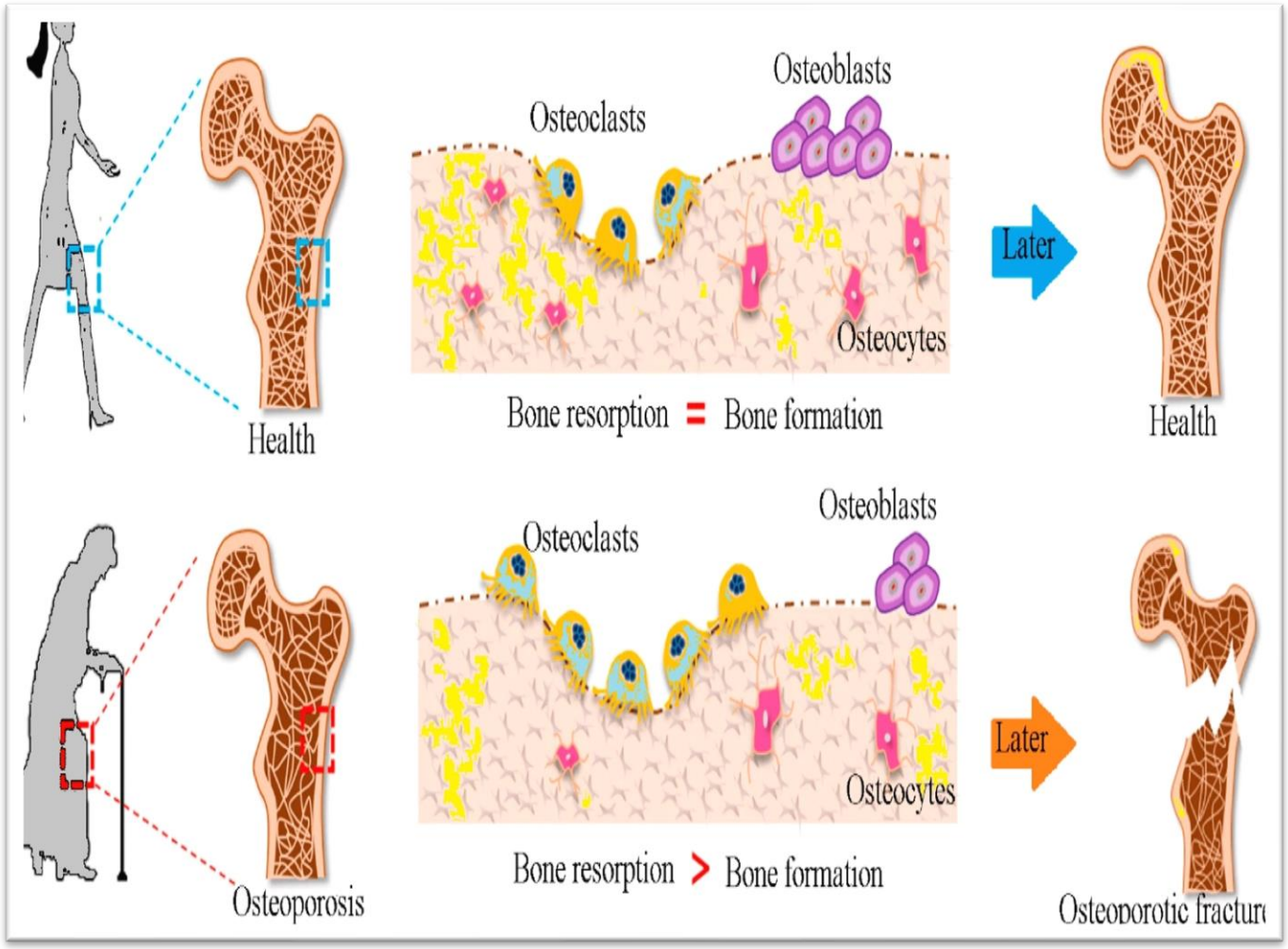

Fig. 1.2: Bone resorption in osteoporosis

1.4 SPECTRUM OF BONE DISEASES ALONG WITH EFFECTS OF ANTI-EPILPETIC DRUGS

A substantial amount of research suggests a link between AED use and abnormalities of the bone, including problems with bone mineral metabolism (Andress DL et al., 2002).

An elevated risk of fracture is associated with a decline in BMD. Bone abnormalities have been shown to exist both radiographically and histologically using bone biopsies and dual energy X-ray absorptiometry (DEXA), the gold standard technique. Initially subtle and asymptomatic, bone loss linked to AED usage generally remains unnoticed for extended periods and is frequently ignored (Nakken KO, Tauboll E., 2010).

The use of AEDs has been linked to a number of biochemical abnormalities, including hypophosphatemia, hypocalcemia, decreased serum levels of vitamin D (biologically active metabolite), and elevated parathormone (PTH) levels. C terminal extension, osteocalcin, and alkaline phosphatase (Pack AM et al., 2004).

There is an increase in the markers of bone turnover, such as alkaline phosphatase, osteocalcin, and C terminal extension peptide of Type I procollagen, and in the markers of bone resorption, such as hydroxyproline and cross-linked carboxyterminal telopeptide of human Type I collagen. (Meier C, Kraenzlin ME, 2011).

In the late 1960s, bone problems in adults and children were initially linked to antiepileptic medications (AEDs). The most severe forms of these illnesses include fractures, osteomalacia, and osteopenia/osteoporosis. Bone disease has been reported in a number of AED-using patient populations. AED-associated bone disease has been found to be more common in certain populations, including institutionalized patients, postmenopausal women, elderly men, and children.

Patients on AEDs have bone disease as evidenced by radiological and histological examination. There have also been numerous reports of biochemical anomalies related to bone metabolism. It is believed that the length of time spent on AEDs and the quantity of AEDs used are related to the severity of bone and metabolic problems. During monotherapy, phenytoin, primidone, and phenobarbital (phenobarbitone) AEDs are most frequently linked to altered bone metabolism. As of right now, there have been no reports of changed bone metabolism in patients on the newer anticonvulsants (gabapentin, lamotrigine, topiramate, and vigabatrin).

In adults and children, antiepileptic medications (AEDs) were initially linked to bone abnormalities in the late 1960s. Osteomalacia, fractures, and osteopenia/osteoporosis are the most severe symptoms of these illnesses. For instance, postmenopausal women who take AEDs and those who have epilepsy had greater incidence of hip fractures. Furthermore, when comparing institutionalized individuals with epilepsy to a "normal" population, fracture rates are generally higher. This was also observed in a study involving ambulatory epileptic men and women, whose median age was 43 years, and a randomly selected control group.

A correlation between the use of AEDs and bone disease has been identified in a number of patient populations. AED-associated bone disease is more common in institutionalized individuals, postmenopausal women, elderly men (over 47 years of age), and youngsters.

Early data show that institutionalized patients receiving AEDs had higher rates of osteomalacia and rickets. The greatest early study included English individuals who had been institutionalized and discovered florid osteomalacia on bone biopsy in addition to lower serum calcium levels. Clinical research has shown a significant rise in the anticipated fracture rate in institutionalized epileptic patients receiving AEDs. It is challenging to attribute the impact of AEDs to bone disease in institutionalized individuals due to their inactivity, poor food, and lack of sun exposure.

Peak bone mineral density (BMD) is determined in childhood and adolescence and is impacted by hormonal, genetic, and exogenous variables. Some exogenous factors that negatively impact BMD

are physical limitations, smoking cigarettes, inadequate calcium intake, and some drugs. For this reason, children are especially vulnerable to the long-term negative effects of drugs on bone metabolism. Several studies have shown that children receiving AEDs had a much higher incidence of hypocalcaemia and signs of decreased bone density.

To evaluate the health of bones, several methods have been employed such as bone biopsy, histo-morphometric analysis, X-ray, single photon absorptiometry, dual-energy Xray absorptiometry, and ultrasound. Biologics were used in early research to better understand how AEDs affected bone. In recent times, there has been a focus on assessing bone mass by various methods, such as ultrasound and basic radiography.

Many methods have been employed to identify reduced bone mass in AED-using patients. In early trials, children with epilepsy using AEDs showed radiographic alterations consistent with reduced mineral content. Simple radiography, however, is unable to detect variations in mineral concentration of less than 30 to 60%. Then, single photon absorptiometry became more well-liked since it is more exact and accurate, and radial locations showed bone density decline.

Single photon absorptiometry is not the best method for diagnosing skeletal diseases because its application is restricted to the peripheral skeleton. Dual-energy X-ray absorptiometry, one of the most recent technologies, examines the total mineral content, evaluates trabecular bone (the spine and ribs), and can identify a 5% or less loss of bone mass. Several studies have used dual-energy X-ray absorptiometry to determine bone mineral density (BMD) in adult patients on antiemetic drugs (AEDs). The results show dramatically lower density in the femoral neck, whole hip, and spine.

Patients using AEDs have many bones metabolism biochemical abnormalities. The most frequent problems include decreased serum levels of physiologically active vitamin D metabolites, higher serum alkaline phosphatase levels, hypophosphataemia, and hypocalcaemia. Enhanced blood alkaline phosphatase levels, hypophosphataemia, and hypocalcaemia are biochemical markers linked to osteomalacia.

AEDs have an impact on two crucial aspects of bone metabolism: the body's phosphate content and calcium balance. With a reported prevalence ranging from 3 to 30%, hypocalcaemia is a common finding linked to AEDs. For patients on AED polytherapy, the incidence of hypocalcaemia is even higher—it is 30% higher. Additionally, patients who are prescribed AEDs have also been reported to have lower serum phosphate levels.

For healthy bone formation and maintenance, vitamin D is necessary. Vitamin D comes in different forms: vitamin D2, which is derived from food, and vitamin D3, which is created in the skin through a process called photolysis driven by UV light. The liver and kidneys are where vitamin D metabolism takes place.

In the liver, Vitamin D which is first converted into 25(OH) or into 25-hydroxy-vitamin D. This is further converted into 1,25 di-hydroxyvitamin D by addition of hydroxylation in the kidney. The indicator which is used more commonly for vitamin D status is the level of 25(OH) D in serum and reduced level has been seen in both children’s and adults that are using these AEDs. Phenytoin,

carbamazepine, phenobarbital are the specific drugs that are linked to lower the 25(OH) D level in patients that are having polytherapy with lowest levels.

The parathyroid gland secretes parathyroid hormone (PTH), which is directly responsible for controlling calcium levels. PTH serves to enhance bone resorption or disintegration in response to a drop in serum calcium levels. There have been reports of an increase in circulating PTH levels linked to AED medication; one study found that PTH levels were higher in males (26%) and women (42%) when compared to controls.

The most widely used indicator of bone growth is alkaline phosphatase, which has been shown to rise in both adults and children on AEDs. However, as a measure of bone disease, blood total alkaline phosphatase level lacks sensitivity and specificity because it is derived from liver, bone, and other sources. When looking for signs of increased bone turnover, assays that distinguish between isoenzymes in the liver and bone are more sensitive than measuring the overall alkaline phosphatase level. According to studies that evaluated the isoenzymes, the bone fraction was primarily responsible for the patients' elevated levels of total alkaline phosphatase.

Osteoblastic cells release procollagen type I molecules. Extension peptides are produced by further cleavage of the amino terminal and carboxyterminal. One potential blood indicator of bone development is PICP. In one study of patients receiving AEDs, there was a significant increase in PICP in both men and women; in a different study, PICP was elevated in young male patients who had just begun carbamazepine therapy.

Primidone, phenobarbital, and phenytoin are the AEDs most frequently linked to reduced bone density and altered bone metabolism. These three drugs were the first to be linked to bone damage in individuals using AEDs in the late 1960s and early 1970s. The biochemical results show decreased levels of calcium, phosphate, and 25(OH)D, increased levels of osteocalcin and alkaline phosphatase, markers of bone production, and elevated levels of urine hydroxyproline and serum ICTP, indications of bone resorption.

Regarding a correlation between carbamazepine and bone disease, there are contradictory findings. While some research finds biochemical anomalies that are consistent with disruptions in bone metabolism, other studies find no appreciable changes in key markers of bone metabolism, such as 25(OH)D.

Compared to monotherapy, polytherapy using AEDs has been demonstrated to increase the risk of anomalies in bone metabolism. Polytherapy is linked to biochemical alterations such as decreased levels of calcium and 25(OH)D and increased levels of alkaline phosphatase. as undergoing polytherapy treatment, the biochemical parameters of institutionalized epileptic patients exhibited more severe modifications as compared to both non-institutionalized and institutionalized populations without epilepsy. When polytherapy was administered, outpatients with epilepsy also had more severe biochemical abnormalities.

Vitamin D may be metabolized to inactive metabolites by AEDs that stimulate hepatic CYP enzymes. To have a biological impact, vitamin D needs to be activated because it is inactive. Oxidation is the primary process that catabolizes vitamin D molecules, and each step in the

oxidative process results in a steady loss of biological activity. The last 1,25(OH)2D cleavage product is calcitroic acid, a chemical that is inert to life. Numerous enzymes, including several CYP enzymes, are involved in these processes. According to research on both humans and animals, CYP enzyme-inducing AEDs promote the conversion of vitamin D to polar inactive metabolites in the liver microsomes, which lowers the amount of vitamin D that is accessible.

The way bone cells operate may be directly impacted by AEDS. The medications may obstruct calcium absorption through the intestines. Hypocalcemia from impaired absorption would result in PTH hypersecretion as feedback. In one investigation, the effects of phenytoin and phenobarbital on intestinal calcium transport were examined in rats. Rats administered phenobarbital exhibited calcium absorption comparable to controls, while rats treated phenytoin had a significantly reduced calcium absorption rate.

One theory about the potential mechanism of AED-associated bone disease is hyperparathyroidism. There was evidence of hyperparathyroidism in both male patients taking AEDs and those with normal vitamin D status, as well as in men and women with epilepsy receiving AEDs and having a vitamin D deficiency. Through the coupling phenomenon, hyperparathyroidism can firstly stimulate bone resorption and then secondary stimulate bone growth. However, enhanced bone turnover (elevated indicators of bone production and resorption) was observed in a recent study of people receiving carbamazepine [48] in the presence of normal vitamin D chemistry and normal PTH levels.

There are several treatment options for bone disease, such as calcitonin, bisphosphonates, hormone replacement therapy, high-dose calcium, and vitamin D supplements. Few trials, nevertheless, have assessed the effectiveness of these treatments in bone diseases linked to AEDs. In one study, vitamin D supplementation was administered and prospective monitoring was conducted for institutionalized and non-institutionalized AED-using people with low 25(OH)D levels. Alkaline phosphatase, calcium, and 25(OH)D levels were measured at baseline and at follow-up. In a span of 12 months, 17 out of the 18 patients who were not institutionalized were able to obtain normal levels of 25(OH)D; one patient reached normal levels after 15 months. After a year, all patients who were institutionalized returned to normal. The necessary vitamin D dosages were 400–4000 IU per day.

Lifestyle modifications can aid in the prevention and treatment of AED-associated bone damage in addition to pharmaceutical therapy. These consist of a healthy diet, exercise, and exposure to sunlight. AED-related bone damage was first reported in large numbers in institutionalized people, and it's possible that these patients' low vitamin D levels had something to do with it. Additionally, all epilepsy sufferers must to receive counseling on preventing accidents. Preventing falls during seizures could potentially mitigate the severe effects of bone damage associated with AED use.

1.5 EFFECTS OF ESTROGEN, TGF-3 AND AEDS ON BONE

In general, there are many people who suggests that estrogen and progesterone help to keep bones strong.

Together with parathyroid hormone's effects on calcium, which estrogens block, cytokine production also occurs. Furthermore, by controlling platelet-derived growth factor (TGF3), a bone matrix protease with anti-osteoclastic qualities, they facilitate the increasing involvement of osteocytes (Robinson et al., 1996).

It is important because it balances the growth of bone matrix by osteoblasts and the removal of bone matrix by osteoclasts. This keeps the density of bone stable. Also, estrogen deprivation has been shown to cut down on the amount of TGF- in rat bones.

1.6 ESTROGEN AND EPILEPSY

Estrogens, among other sex hormones, are crucial to the reproductive system's operation. Estriol (E3) is believed to be the most physiologically active version of all three physiologically active forms of estrogens: estrone (E1), estradiol (E2), and estriol (E3). Estrogen has a well-known neuromodulatory effect and is believed to regulate several pathways in the brain to regulate its shape and function (McCarthy, M.M.,2008).

Postmenopausal women have a higher frequency of neurological illnesses, which may be related to the role of estrogen and its removal after menopause (Christensen A, Pike C.J., 2015).

Following menopause, estrogen replacement therapy has been shown to postpone the start and pathogenesis of Parkinson's, Alzheimer's, and stroke diseases. Moreover, it has been noted that the most prevalent neuropathology linked to temporal lobe epilepsy is the death of hippocampus neurons (de Lanerolle et al., 2003).

1.7 ESTROGEN AND BONE

Within four years of beginning, peak bone mineral density is gained due to the pubertal rise in steroid release, especially estrogen, which promotes the mineralization of skeletal tissues. For the ten years following menarche, it also stimulates the longitudinal and radial expansion of skeletal tissue in addition to mediating bone mineralization through sex steroids.

By the action of estrogen & progesterone receptors on the bone cells that circulates estrogen but to a lesser extent in which progesterone directly governs its skeletal development and mineralization. By the estrogen receptors on the bone cells which has been shown in animal’s studies that directly stimulate the creation of bone (Khalid A.B., Krum S.A., 2016).

Consequently, a lack of estrogen is likely to impair bone health and may cause the loss of mineralization and formation of new bone. In addition to promoting the growth and mineralization

of bones, estrogen also inhibits the resorption of bone, which helps women's bone mass rise from puberty until menopause. The positive effects of circulating estrogen levels on bones are compromised by diminished ovarian function following menopause, which increases the risk of fracture and significantly lowers bone mineral density.

The oestrogen deficiency increases interleukin -7 production that further encourages T cell release of interferons & the removal of its anti-oxidant further shows its impact on the bone cells. The released interferons & produced free radicals accelerate the production of RANKL & tumor necrosis factor which further promotes osteoclastogenesis by attracting the osteoclasts & decreases the death of osteoclastic cells (He Xin et al., 2016).

Research indicates that osteoblastic cells, T cells, and B cells express RANKL, a determinant of osclastogensis, less frequently when estrogen is present. Osteoblastic cells and stromal cells of the bone marrow also express osteoprotegerin (OPG), a soluble glycoprotein (Jia J et al., 2017). OPG inhibits bone resorption by preventing osteoclast cells from differentiating and by preventing RANKL from interacting to the RANK receptor that is expressed on osteoclast precursor cells.

As a result, an estrogen shortage causes RANKL expression to rise, and a decrease in OPG expression encourages RANKL and RANK binding, which in turn promotes osteoclastogenesis and bone resorption. Moreover, estrogen controls the production of several cytokines from bone marrow-derived osteoblast and stromal blood cells, which in turn affects osteoclastic bone resorption (Khosla S et al., 2002).

We hypothesize in this study that AED medication-induced estrogen depletion could have detrimental effects on bones. Reasons for our faith in the same are as follows:

- The human aromatase (CYP19) enzyme, which prevents testosterone from being converted to estradiol, is blocked by many AEDs.
- AEDs make mitochondria break down estradiol and estrone, which lowers growth hormone and other gonadal androgens that are turned into estrogens. This reduces growth hormone and other testicular androgens that are turned into estrogens.
- A vitamin D deficiency caused by an AED could lead to less androgen receptor manufacturing, leading to bone problems through a) as previously explained.

1.8 DRUGS PROFILE

1.8.1 VITAMIN D & CALCIUM

The use of Vitamin D & calcium is accepted baseline treatment for the disease osteoporosis. In a clinical study that is conducted for 3 years there were supplementation of calcium & Vitamin D3 reduced the risk of hip fractures & other non-vertebral fractures in elderly women which was further beneficial after 18 months (Chapuy MC et al., 1992).

Bone health is dependent on vitamin D levels. Given the extensive distribution of vitamin D receptors in numerous tissues, it is possible that vitamin D has a variety of physiological functions (Rosen C.J et al., 2012).

A lack of vitamin D has also been linked to osteoporosis, rickets, cancer, autoimmune diseases (like multiple sclerosis and rheumatoid arthritis), chronic fatigue, depression, falls in the elderly, diabetes, vascular diseases (like heart disease and stroke), neurodegenerative diseases, and epilepsy (Deluca H.F.,2004).

Since peak bone mass is often reached by the age of 30, ensuring optimal bone mass development during adolescence and early adulthood through physical exercise and taking the required dosages of calcium and vitamin D (IOM, 1997).

In the human body, calcium is a vital component that is required for numerous cell processes. In addition to being crucial for the health of bones, calcium is also required for blood clotting, neuromuscular activity, and regular heart function. It is essential to the construction of bones and necessary for the lifetime deposition of bone mineral. The extracellular fluid (ECF), sometimes known as plasma, contains calcium in addition to the bones and teeth, where the body stores more than 99% of it. The calcium balance is determined by plasma calcium levels.

The consumption of foods high in calcium content is the most effective method of fulfilling the daily dietary requirement. Because dairy products have a high calcium content, a high absorptive rate, and are very inexpensive, they are the best sources of calcium. Dairy products (milk, cheese, yogurt) and some green vegetables are dietary sources of calcium.

The number of dairy products ingested every day is about 300 milligrams. One cup (8 ounces) of milk, one cup of yogurt, or one to 1.5 ounces of cheese constitute a serving size of dairy products. Thus, an estimated total elemental calcium consumption would be obtained by multiplying each daily dairy item by 300 mg.

Another source of dietary calcium is calcium-enriched mineral waters. According to a recent study, high-calcium mineral waters may supply beneficial levels of bioavailable calcium and have absorbabilities that are comparable to or slightly better than milk calcium.

Antiepileptic medications (AEDs), in particular enzyme-inducing AEDs (EIAEDs), have been linked to a lower risk of fracture and decreased bone mineral density. The way that AEDs affect the metabolism of vitamin D may be one significant cause.

To satisfy these recommendations, people who don't get enough calcium from their diets should take a supplement. There is evidence to imply that the average American does not consume the necessary amount of calcium per day. Less than half of the recommended calcium intake for postmenopausal women is consumed by ordinary women over 40. In one study, 82% of osteoporosis patients were not taking the required daily dose of 1000 mg (Black et al., 1996).

It has come into knowledge that there are many easily found calcium supplements that ensures proper intake of minerals. Calcium carbonate & calcium citrate are the two most popular &

completely researched calcium supplements. When they are taken with meal, both the supplements have been seen to have equal absorbative property.

1.8.2 BIPHOSPHONATES

The most widely used medications for osteoporosis treatment are bisphosphonates. They attach themselves to bone with vigor, and osteoclasts take them up to prevent resorption. They come in two classes: potent nitrogen-containing bisphosphonates and low potency non-nitrogen-containing bisphosphonates. They can be given orally or intravenously. These two classes have distinct intracellular targets and molecular mechanisms of action that lead to inhibition of osteoclast-mediated bone resorption (Rogers MJ et al.,2011).

The known phosphate -carbon-phosphate backbone of all the bisphosphonates have two side chains R1 & R2. The one non-nitrogen containing bisphosphonates are metabolized into osteoclasts into non-hydrolysable analog of adenosine triphosphate that accumulates and triggers osteoclasts apoptosis. The simple side chains are CH3 groups in etidronate & Cl groups in clodronate (Frith JC et al., 2001).

Alendronate (Fosamax®; oral, 70mg) the most commonly prescribed drug used in the treatment of post-menopausal osteoporosis which is associated with increased BMD & fracture risk reduction.

Osteopenia in the growing rats was not seen after giving an active form of Vit. D or bisphosphonate with VPA. Then animal data on bisphosphonates documents its preventive & therapeutic effects when further giving PHT as 35mg/kg for 3 months or giving it after PHT-induced bone loss in mice (Khanna S et al., 2011).

Bisphosphonates are administered intravenously or orally. When bisphosphonates attach to the surface of the bone mineral, they can effectively block the resorption of bone mediated by osteoclasts. They then embed in the bone and release their effects only when the bone resorbs.

Bisphosphonates that have the highest binding affinity to bone (zoledronic acid > alendronate > ibandronate > risedronate) may remain in bone longer than other antiresorptive medications. As a result, patients may continue to experience the pharmacologic effects of these medications for years after stopping them.

Oral or intravenous administration is used for bisphosphonates. In optimal circumstances, around 1% of the dosage taken orally is absorbed. The skeleton absorbs up to 50% of the ingested or intravenously delivered dose very fast. The remaining medication is excreted by the kidneys without being metabolized. In skeletal tissue, bisphosphonates have a lengthy residual half-life (years). The medication that is left in the skeleton has very little effect since it is hidden by growing bone and kept apart from osteoclasts.

When bone is undergoing active remodeling, bisphosphonates preferentially attach to its surface and integrate into osteoclasts. Bisphosphonates that do not contain nitrogen, such etidronate and clodronate, prevent bone resorption by producing a hazardous analog of adenosine triphosphate

that disrupts mitochondrial activity and causes osteoclasts to undergo apoptosis. The strong nitrogen-containing medications in this family, such as zoledronic acid, alendronate, risedronate, ibandronate, and pamidronate, inhibit the distal step in the cholesterol production pathway called farnesyl diphosphate synthetase.

When bisphosphonates are taken orally, biochemical markers of bone resorption are suppressed, reaching a sustained nadir of 50–70% below baseline after three months, or around 50% of baseline after one month. The rate of bone production decreases and stabilizes between six and twelve months of treatment. In general, bone turnover is decreased to the levels observed in young people in good health (Miller PD et al., 2005).

Patients with osteoporosis see a clinically significant decrease in the frequency of both vertebral and non-vertebral fractures, such as hip fractures, when they get bisphosphonate medication. After starting treatment, fracture protection happens a few months later and lasts for at least a few years. Significantly, there is a 77–96% decrease in the frequency of progressive, multiple spinal fractures.

Since they are all poorly absorbed from the GI tract & attach to many kinds of food, beverages and other medications. It should be known that we have to take them on an empty stomach with 4-8 ounces of normal water at least 30 minutes before taking any food, beverages or any kind of medication. To reduce the reflux & known GI problems the patient should not lay down for around 30 minutes after taking the medicine. Ibandronate is given through IV as a bolus injection (15-30 minutes).

1.8.3 SELECTIVE ESTROGEN RECEPTOR MODULATORS

These chemically varied molecules lack the steroid structure of estrogen, but instead have a tertiary structure that enables them to attach to the estrogen receptor and act as selective agonists or antagonists on various tissues that are targets of estrogen. The most studied SERM is raloxifene, which reduces vertebral fractures in osteoporotic women; however, it did not significantly decrease the risk for non-vertebral or hip fractures compared with placebo (Komm BS, Chine AA,2012). While raloxifene can lower the risk of breast cancer, there is a chance that it will increase the risk of thromboembolism, leg cramps, stroke, and postmenopausal vasomotor symptoms. Hot flushes may worsen and there may be a higher risk of venous thrombosis, comparable to that of hormone therapy.

1.8.4 CALCITONIN

Calcitonin is a polypeptide hormone called calcitonin significantly reduces osteoclast activity via a process controlled by receptors. It acts on a G protein-coupled calcitonin receptor, which primarily transduces signals via the cAMP and PLC/IP3 pathways (Zaidi M et. al.,2002). The kidney and hypothalamus both exhibit significant levels of calcitonin receptor expression. The receptors in bone are mostly found in the osteoclast membranes, which inhibits the osteoclasts' ability to move freely and resorb bone. It stops the maturation of osteoclast precursors. Carbonic anhydrase II is also inhibited, which ruins the acidic environment that is ideal for osteoclast activity.

1.8.5 SODIUM VALPROATE

Valproic acid was found to stop seizures for the first time in 1963, when it was being used as a solvent for other chemicals. The sodium salt was made into an anti-seizure drug in 1967, and the UK started selling it in 1974. Over the past ten years, there has been slow but steady growth in the number of types of seizures for which sodium valproate (SVP) has been shown to be helpful. Valproate is being used more and more around the world now that we know more about how dangerous it is. So, now is a good time to take another look at the youngest of the three first-line anticonvulsant drugs.

One of the most often used antiepileptic (anticonvulsant) drugs (AEDs) for treating various forms of epilepsy is valproic acid, also known as valproate. It is a significant and well-established first-line AED. The trivial name for 2-n-propylpentanoic acid, often known as n-dipropylacetic acid, is valproate. As a basic fatty acid with branched chains (Löscher W. Valproate,1999).

Sodium valproate is a white powder that tastes salty and has no smell. It dissolves very well in both water and alcohol. It has a weight of 166 moles. It is very different from well-known anticonvulsants like barbiturates, hydantoins, succinimides, oxazolidinediones, and acetylureas because it doesn't have any nitrogen or aromatic molecules.

1.8.6 PHENYTOIN

It was discovered in the 1970s that there was significant variation in the bioavailability of phenytoin across preparations, which could lead to issues when one phenytoin preparation was swapped out for another. Phenytoin is a weak acid (pK, 9) and only sparingly soluble in water. It can be used as an acid or as a sodium salt, which changes into an acid at the pH of the stomach. Tablets and powders are the pharmaceutical forms that are used to make injectable solutions. The dissolving duration of the tablets and variations in the raw material's particle size are two factors that can affect the bioavailability of parallel preparations. The therapeutic impact of these discrepancies is further enhanced by the saturation kinetics of phenytoin. Additionally, the rate of absorption differs between preparations and patients. (Neuvonen P.J., 1979).

There are many adverse effects such as drowsiness, fatigue, dizziness, disorientation, impaired vision, nystagmus, and tremors are the most often reported adverse effects (CNS effects). Negative effects on the digestive tract include feeling sick, throwing up, and losing your appetite. Additionally, it can lead to death (> 100 mg/L), decreased mental activity (> 40 mg/L), and far lateral nystagmus (> 20 mg/L). Other phenytoin side effects, such as gingival hyperplasia, hirsutism, facial thickness, vitamin D deficiency, folic acid shortage, osteomalacia, and peripheral neuropathy, are not dose-related (Zeng K et al., 2010).

1.9 THE PROCESS AS HOW THESE AEDS AFFECTS BONE HEALTH?

The methods by which AEDs deplete bone are intricate. The predominant theory for many years was that AEDs that induce enzymes, such as phenobarbital, phenytoin, carbamazepine, and oxcarbazepine, accelerated the hydroxylation of vitamin D to polar inactive metabolites. This, in turn, caused increased bone turnover and higher rates of bone loss, as well as hypocalcemia and secondary hyperparathyroidism (Fitzpatrick LA., 2004). Moreover, medications that do not stimulate CYP-450 hepatic enzymes, like valproate, have been demonstrated to have an impact on bone health.

Other hypothesized causes include decreased intestinal absorption of calcium, resistance to PTH, calcitonin insufficiency, disruption of vitamin K metabolism, and direct pharmacological effects on bone cell activities (Feldkamp J et al., 2000). Additional, unintended consequences of the medications could include changes in hormones (such as hypogonadism), an increase in homocysteine, a decrease in IGF-I, and the action of medications like VPA as an HDAC inhibitor.

1.10 OSTEOPOROSIS

The disease osteoporosis that is due to low bone mass and deterioration of bone tissue with further disruption of bone mini architecture that leads to compromised bone strength and increase in the chances of fractures (JAMA, 2001).

The highly accumulated bone disease in humans is primarily osteoporosis, which is a very serious health issue. It is more accommodated in older adults, women and Caucasians. As the hypertension increases the chances of stroke similar to it this disease increases the chances of fractures. Many peoples of all the ages and genders are suffering from this disease and all the population progresses so its prevalence of the condition increases. It is a disorder that remains detached until the fracture takes place that further leads to very serious health issues and sometimes even death. It was estimated that the number of patients around the globe with osteoporotic hip fracture are more than 200 million (Cooper C et al., 1992).

On global basis the number of older adults has further increased as a result of longer life spans. In India, the average life expectancy is currently 67 years but on prediction basis it has rinsed to 71 years by the time of 2025 and 77 years by 2050. Regularly low calcium is taken as seen in toddlers, adolescents, pregnant mothers and also in postmenopausal Indian women's.

The overall risk of fractures in the hip, wrist, spine is around 40% & the lifetime risk of hip fractures in women is around 14% with risk it increases as the age continues. At 80 years of age around 20% of women have hip fracture & at 90 years around 50% of women have hip fractures. The women who are more age than 85 years & are 8 times more induced than the women of 65-74 years of age for the going to the hospital in case of hip fracture. In addition to it, there is more chance of higher morbidity & mortality of the patients who are suffering from osteoporosis (Kaushal N et al., 2018).

In postmenopausal women, there are two stages to bone loss. Menopause-related bone loss happens quickly during the first three to five years of the short phase, while age-related bone loss causes both men and women to gradually lose their cortical and trabecular bones over the course of more than ten to twenty years. The most frequent clinical outcomes of osteoporosis are fracture, incapacity, and chronic pain. The most frequent osteoporotic fractures are those of the pelvis, vertebrae, and distal radius.

Assessing the risk of fracture is aided by BMD measurement. Studies in the future have shown that a decline in BMD is linked to a higher risk of fracture. Measuring BMD, however, might not be able to identify all women who are fracture-prone. Less than half of the breakages in two sizable cohort studies happened in women whose T-scores were less than or equal to -2.5. Treatment decisions are not usually based only on BMD data. In particular, the risk of falling must be taken into account along with other risk factors.

Fractures coming from mechanical forces that would not be a normal cause of fracture is known as osteoporotic fractures. The osteoporotic fractures that include fractures of the spine, forearm, hip, shoulder ate those which are connected to low mineral density (BMD).

The reduction in estrogen levels brought on by menopause is the main factor contributing to the pathophysiology of postmenopausal osteoporosis. Albright et al. first put forth this theory in the 1940s, when their groundbreaking research showed that osteoporosis in postmenopausal women had a negative calcium balance, which could be corrected with estrogen replacement therapy. Albright and colleagues thought that estrogen shortage-induced poor bone formation was the primary cause of bone loss in postmenopausal women; however, later research revealed that estrogen insufficiency mostly causes an increase in bone resorption. Nonetheless, it is well known that postmenopausal osteoporosis results in increased bone resorption as well as increased bone formation; however, the amount of increased bone resorption is greater than the amount of increased bone formation, leading to an imbalance in favor of increased bone resorption (Erikson EF et al., 1990).

As it was determined that the insufficiency of estrogens shows a major role in the pathophysiology of osteoporosis, that is a major deal of research which undergoes for the mechanism by which estrogen protects the bone. By understanding the molecular & cellular mechanism that are pinned by the involvement of estrogen insufficiency as the etiology of post-menopausal osteoporosis has gained advancement in the past three decades. There was research conducted in the 1980s that showed that a wide range of cytokines for example, interleukin (IL)-1,6,7; tumor necrosis factor, M-CSF, regulated the differentiation & activity of osteoclasts. Then it develops a question of whether estrogen prevents osteoporosis by the influence of cytokine production. The estrogens actually inhibit the expression of IL-1, YNF & IL-6 in monocytes, osteoblasts & stromal cells as per the research on in-vitro & in-vivo.

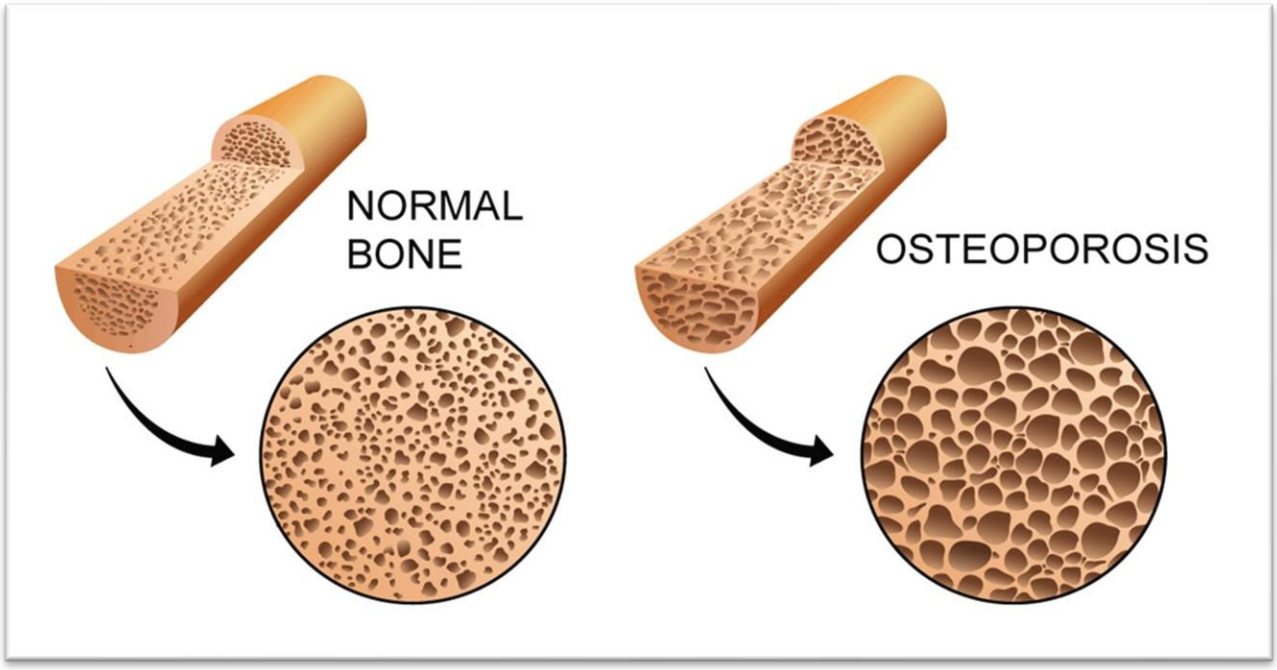

Fig. 1.3: Normal bone vs Osteoporosis Bone

1.10.1 EPIDEMIOLOGY

One of the main risk factors for fragility fracture is decreased bone density. Additional variables that could impact the likelihood of a fragility fracture include age, gender, history of fractures, usage of oral or systemic glucocorticoids, and familial history of osteoporosis. The prevalence of osteoporosis rises sharply with age in women, rising from 2% at 50 to over 25% at 80 years old due to accelerated bone loss following menopause and age-related bone loss in both genders.

Antiepileptic medications (AEDs) are used by the majority of the 50 million persons who have epilepsy worldwide. But the usage of AEDs is rising steadily, even among persons who do not have epilepsy. Thus, it's interesting to see how AED use and osteoporosis are related.
The association between using an AED and fractures is debatable; some research, but not all of them, indicate a higher risk of fractures. The sample size, choice of controls, and—most importantly—the omission of potentially confounding variables are the limitations of these reports. The Women's Health Initiative (WHI) contains a large pharmacoepidemiologic database that looked at the relationship between AED use and fractures, but it was unable to account for significant potential confounders like smoking history, family history of fractures, and calcium/vitamin D intake (Vestergaard P et al., 2004).

AED use and fractures may be related through a variety of pathways. AED use has been linked to vitamin D shortage, and enzyme-inducing AEDs (cytochrome P450 enzyme system inducers) in particular may be linked to vitamin D deficiency due to the possibility of increased vitamin D

catabolism caused by enzyme induction. Moreover, osteocalcin, a vitamin K-dependent protein that is a particular byproduct of osteoblasts, has been linked to AEDs under carboxylation of the bone Gla protein.

This could be significant because epidemiologic research points to a link between higher fracture rates and osteocalcin's under carboxylation. The history of falls is significant since they can be linked to AED use and/or the disease for which an AED is given. Fall risk is impacted by the most prevalent side effects of AEDs, which include dizziness, ataxia, and unstable walking. On the other hand, AEDs are used to treat ailments including diabetic neuropathy and seizures, which can lead to falls (Wirrell Ec., 2006).

The most significant aspects of osteoporosis are its shape and the role of the elderly and postmenopausal women. Osteoporosis is linked to both early menopause (between the ages of 40 and 50) and premature menopause (before the age of 40). The bone density in later life stages will decrease with an earlier menopause. Women who had their ovaries surgically removed before turning 45 are more likely to develop osteoporosis.

Early childhood physical and nutritional intervention, management of risk factors like smoking, timely treatment of hormone imbalance diseases, menstruation, and menopause, and necessary replacement therapy are the first steps in prevention.

Based on measurements of bone mineral density (BMD) obtained from dual energy x-ray absorptiometry (DEXA) evaluation, osteoporosis is diagnosed radiographically. While peripheral DEXA and quantitative calcaneal ultrasonography can also predict fracture risk, their correlation with central DEXA is not strong enough to be useful in diagnosis. The World Health Organization (WHO) defined osteoporosis and osteopenia according to accepted standards.

Children and premenopausal women should not be subjected to the WHO criteria, nor should men under the age of fifty. The International Society for Clinical Densitometry advises using the z score (age and sex norms) for these populations. Z scores that are less than -2.0 fall outside of the typical age range. A BMD evaluation by itself is insufficient to diagnose osteoporosis in men under 50.

Aging and the decrease of gonadal function are linked to primary osteoporosis. Secondary osteoporosis is brought on by additional medical disorders, In postmenopausal women, secondary causes of osteoporosis are thought to account for up to 30% of occurrences. If vitamin D deficiency is taken into account as a secondary cause, the estimate rises to more than 50% in men, premenopausal women, and perimenopausal women. Expert consensus recommends a basic laboratory assessment for all newly diagnosed patients in addition to a physical examination and history taking. This is to identify common secondary causes and find out if there are any contraindications to any osteoporosis drugs.
The treatment is recommended by the National Osteoporosis Foundation for postmenopausal women & men that have a personal history of hip & vertebral fracture as a T-score of -2.5 or less or a combination of low bone mass of the T score between -1 & -2.5 and a 10-year probability as per FRAX WHO Fracture risk assessment tool as of at least 3% for hip fractures or at least 20% for any other kind of major fractures. WHO recommends that the people who are having osteoporosis or at higher risk of it should take the treatment considerations.

Patients with osteoporosis should prioritize fall prevention because it is more directly linked to fracture risk than bone mineral density (BMD). The USPSTF advises community-dwelling persons 65 years of age or older who are at elevated risk of falling to engage in physical therapy or exercise as well as take vitamin D supplements to prevent falls. It is advisable to suggest a multi-component workout regimen that includes balance training and weight-bearing resistance. Programs for aerobic exercise that do not include strength and balance training should be avoided due to the link to an increased risk of fractures. There is compelling evidence that a comprehensive evaluation of a patient's fall risk and the reduction of associated risk factors can effectively avoid falls.

Oral bisphosphonates are antiresorptive and inhibit osteoclastic activity. They are regarded as the first line of pharmaceutical treatment. Alendronate (Fosamax) and risedronate (Actonel) have been shown in randomized clinical trials to reduce the incidence of hip and vertebral fractures. Risedronate and alendronate also reduce the risk of vertebral fractures in males and in individuals with osteoporosis brought on by glucocorticoids. Ibandronate (Boniva) has been shown to be beneficial in lowering spine fractures alone when used daily or sporadically. Dosing formulations with weekly and monthly intervals enhance adherence. When taking oral bisphosphonates, one should only take them with water and wait for at least half an hour before lying down or consuming any other medications or food. This promotes proper absorption and lessens harmful effects on the upper gastrointestinal tract.

It is unknown how long oral bisphosphonate therapy should last. According to one study, women who take alendronate for five years and then a placebo for a further five years do not experience a higher incidence of hip or nonvertebral fractures than women who take alendronate for ten years. On the other hand, the number of spinal fractures is rising. Atypical femoral fractures and osteonecrosis of the jaw are uncommon side effects of bisphosphonate medication that are linked to extended usage periods. In women who do not have a personal history of vertebral fractures, clinicians should think about stopping bisphosphonate therapy after five years.

Only vertebral fractures can be prevented using raloxifene, a selective estrogen receptor modulator that is authorized for the treatment of postmenopausal osteoporosis. Raloxifene frequently causes worsening vasomotor symptoms. It is linked to a lower risk of invasive breast cancer and an increased risk of venous thromboembolism.

Postmenopausal women with osteoporosis who cannot take bisphosphonates, do not have a history of venous thromboembolism or vasomotor symptoms, and have a high breast cancer risk score are the best candidates for raloxifene. More recently, the United States approved the use of zedoxifene, a selective estrogen receptor modulator, in combination therapy with conjugated estrogen (Duavee) to prevent osteoporosis.

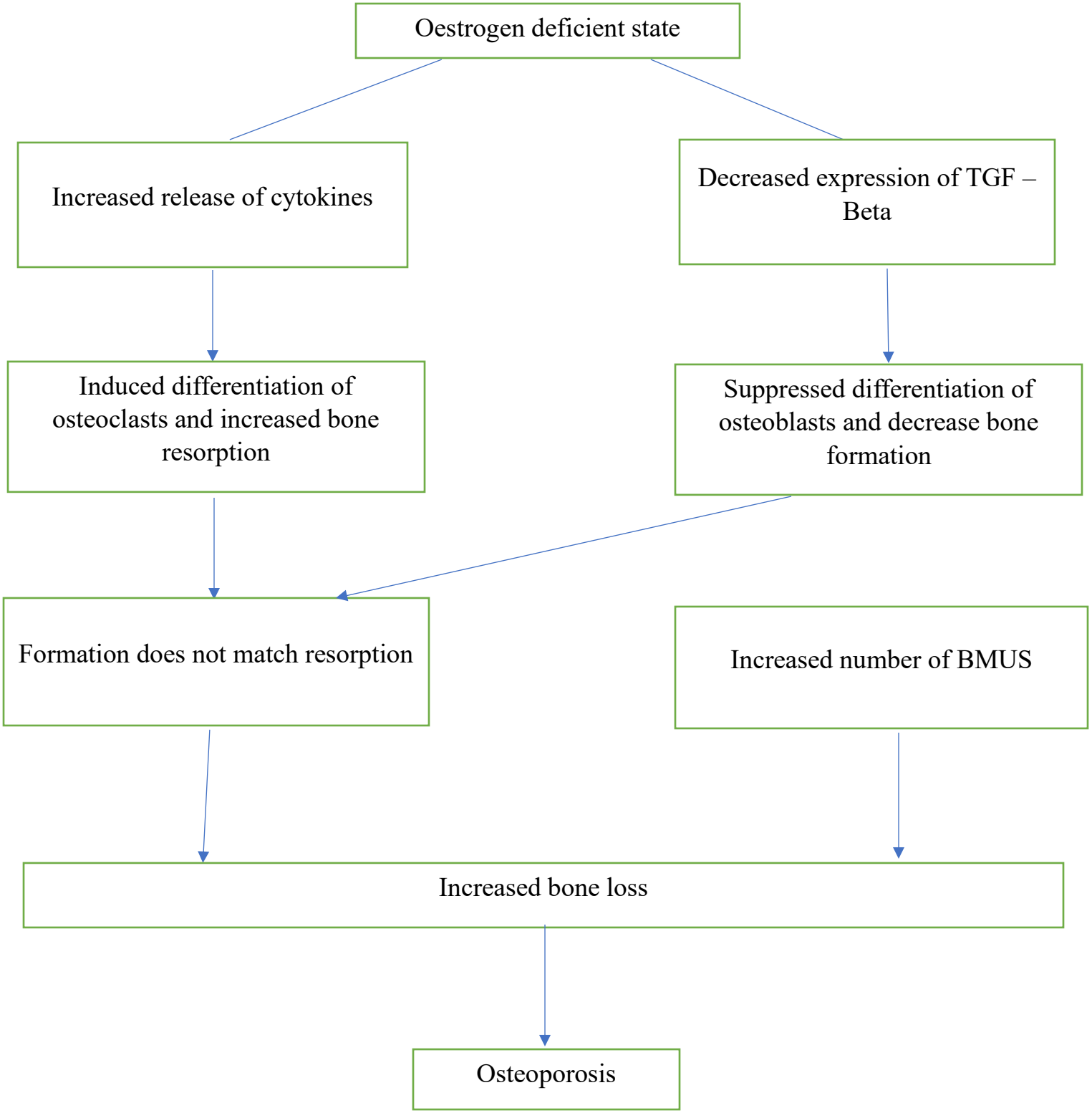

Fig. 1.4: Factors involved in the pathogenesis of osteoporosis

1.11 RISK FACTORS ALONG WITH ITS CAUSES OF OSTEOPOROSIS

It is found that there are many primary & secondary causes of osteoporosis. In which Type-I is also known as post-menopausal osteoporosis which has a linkage with menopause and Type-II is also known as age – related osteoporosis that affects the people over age 70 years. There are certain other factors such as hematological illness, medicines, chronic renal disorder, Gastro-intestinal disorder, connective tissue disorder are all the examples of secondary causes of osteoporosis.

Women typically have smaller frames, consume less foods high in calcium, and receive less solar exposure due to sociocultural factors. Further to it, estrogen which is important for the formation & growth of bone in women, reduced periods of exposure to estrogen at the women's lifetime also contributes to the development of osteoporosis (Parker et al., 2014).

One of the main contributors of osteoporosis is the menopause. Every year, postmenopausal women lose 3%–5% of their bone mass. Following menopause, these women lose a portion of their bone mass and are susceptible to osteoporosis for up to seven years. The ovaries' decreased ability to produce estrogen is the cause of bone loss following menopause. Because women experience decreased bone mass and an increased risk of fractures during one-third of their lives, as well as a high rate of bone loss in the early years after menopause, menopausal osteoporosis is a significant problem.

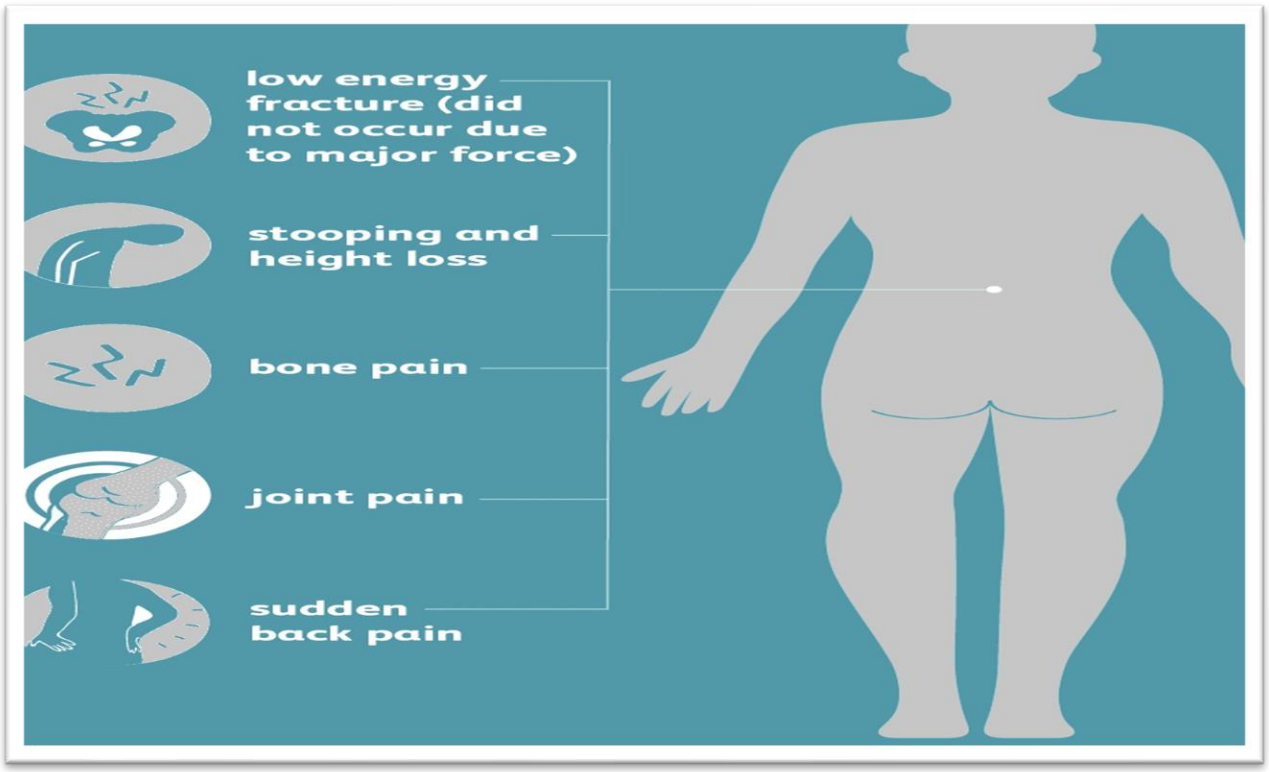

Fig. 1.5: Causes of Osteoporosis in Women

1.12 HOW THE BODY WORK AFFECTS CHANGE IN BONE

There are various cell types that make up the bone remodelling cycle are balanced in their activity; the skeleton remains intact. These are the cells that build the organic bone matrix and facilitate its mineralization, known as bone-forming osteoblasts The bone degrading osteoclast, a unique type of exocrine cell that dissolves bone mineral and enzymatically degrades extracellular matrix (ECM) proteins osteocyte, an osteoblast-derived post-mitotic cell within bone matrix that acts as a mechano sensor and an endocrine cell (Karsenty G et al., 2009).

Osteoclasts on the surface of the bone become activated during a remodelling cycle and resorb bone matrix, creating a defect that osteoblasts fill in. Typically, the cycle takes 200 days to finish. In healthy and normal settings, bone creation will equal resorption, and the amount of bone tissue at the beginning and conclusion of the cycle will be the same due to the highly regulated nature of the bone remodelling cycle and the strong coupling between resorption and formation.

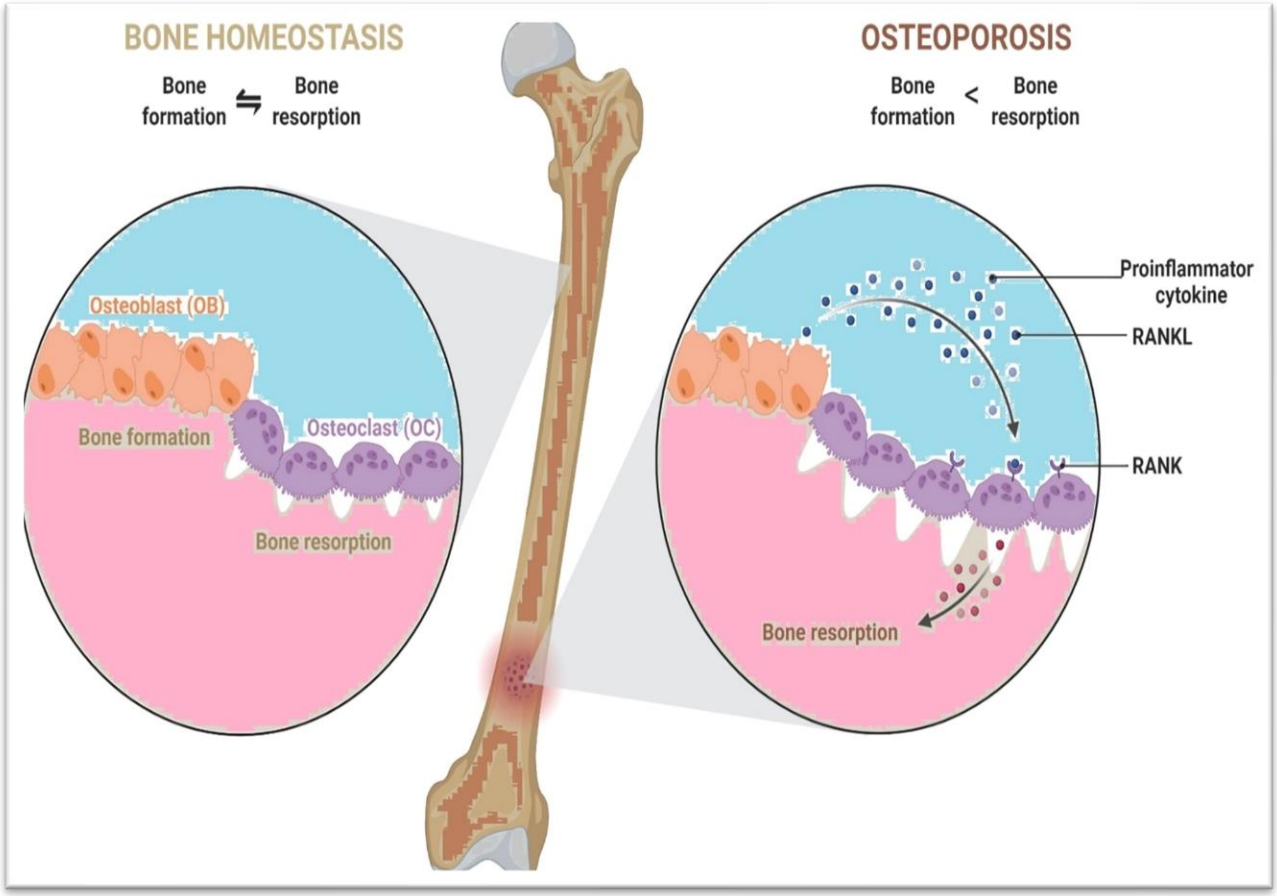

Figure 1.6: Role of Innate Immune cells in osteoporosis

1.13 SKELETAL DEVELOPMENT – BONE ORGANIZATION

Comprising of calcium compounds (hydroxyapatite) crystals, capillaries, and cells, bone is a porous mineralized structure. Depending on the areas and types of bones, their proportion varies. Genes control the processes of cellular differentiation that result in the skeleton. Initially, cartilage and mesenchyme define the pattern of skeletal structure, and subsequently, osteoblast differentiation replaces these materials with bone.

The collagen cells & the extracellular matrix that is mainly mineralized make the structural elements of bone. The complete mature human skeleton that has two different forms of bone; one is trabecular & other is cortical. Insite of having different macro & microscopical features the chemical content of the two forms is the same. 80% of skeleton is made up of cortical bones that is denser and compact has also had a moderate turnover rate with a strong resistance of bending and it also forms the outer layer of all the skeletal structures.

The initial purpose of the calcified cortical bone is to give protection & mechanical strength along with it also shows many metabolic reactions mainly in case of severe or protracted mineral deficiency. 20% of the skeletal mass is made up of trabecular bone but 80% of the bone surface is found inside the long bones that makes vertebral bodies along with-it interior areas of the pelvis & other large flat bones.

Compared to cortical bone, trabecular bone is more pliable, less thick, and has a greater turnover rate, suggesting a significant metabolic role. Particularly in bones like the vertebrae, trabecular bone aids in mechanical support and delivers the first mineral supplies in cases of acute deficit.

1.13.1 BONE MATRIX

About 90% of the organic content of the entire bone tissue is made up of noncollagenous proteins and type I collagen fibers, which are made up of two a1 chains and one a2 chain. The fibers in lamellar bone arrange into arches that permit the maximum amount of collagen per tissue volume. The lamellae might be concentric around a channel centered on a blood artery (the cortical bone Haversian system) or run parallel to each other (the periosteum and trabecular bone).

Hydroxyapatite crystals [3Ca3(PO4)2·(OH)2] are present on the collagen fibers, inside them, and in the matrix. They are generally aligned parallel to the collagen fibers. Many noncollagenous proteins found in the bone matrix have an unclear function. Osteocalcin, also known as Gla protein, is the main noncollagenous protein generated and is involved in calcium binding, hydroxyapatite matrix stabilization, and bone formation control.

Gla protein appears to be a negative regulator of bone development, preventing improper or early mineralization.4 On the other hand, the proteoglycan biglycan, which is expressed in the bone matrix, positively controls the production of new bone.

1.13.2 OSTEOCYTES

Osteocytes are osteoblasts that have been lodged in the osteoid. Once the osteoblast is completely encased in bone matrix, its metabolic activity diminishes, but it still produces matrix proteins. Numerous lengthy cell processes rich in microfilaments are present in osteocytes, and these processes are structured throughout the matrix's development and prior to its calcification. They create a thin canaliculi network that penetrates the whole bone matrix.

The appearance and functional activity of osteoblasts vary with cell age. The majority of the anatomical features of an osteoblast are present in a young osteocyte, but its cell volume and ability to synthesize proteins are reduced. An older osteocyte with a buildup of glycogen in the cytoplasm and a further reduction in cell volume is seen further within the calcified bone. When osteoclastic bone resorption occurs, the osteocytes are eventually phagocytosed and broken down.

The precise role of these cells is still unknown, despite the intricate structure of the osteocytic network. Osteocytes probably attract osteoclasts to places where bone remodeling is needed in response to strain on bone tissue, which increases the activity of bone remodeling. Nevertheless, there is currently no proof that osteocytes communicate with bone surface cells in response to microdamage or bone pressure.

1.13.3 OSTEOBLAST- BONE FORMATION

The components of the bone matrix are produced by the osteoblast. Instead of operating independently, osteoblasts are located in groups along the surface of the bone, lining the layer of bone matrix that they are generating. They develop from multipotent mesenchymal stem cells, which can differentiate into fibroblasts, chondrocytes, myoblasts, osteoblasts, or adipocytes. Recent research on gene deletions has demonstrated that osteoblast development depends critically on the presence of either the downstream factor osterix or the run trelated transcription factor 2 (Runx2).9. 15% of mature osteoblasts are trapped in the new bone matrix and develop into osteocytes at the end of the matrix-secreting phase. Conversely, some cells stay on the surface of the bone and develop into flat lining cells.

The creation with maturation of osteoid matrix that is followed by the matrix mineralization are the 3 stages in which the bone is formed. These processes take place in the same way as in healthy adult bones that ensures that the matrix synthesis & mineralization are balanced equally. The osteoblasts first create osteoid by Fastly depositing collagen, The rate of mineralization then increases to match up the rate of collagen synthesis. The collagen synthesis further slows down in the final stage and mineralization should keep going until the osteoid is fully mineralized.

Insulin like growth factors (IGF), Platelet derived growth factors (PDGF), basic fibroblast growth, transforming growth factor -beta are the growth factors that osteoblasts can create in response to the stimuli. These given growth factors have receptors on osteoblasts, that works in autocrine & paracrine way for regulating osteoblastic activity. These osteoblasts also have receptors for the classic hormones such as growth hormone, insulin, progesterone, prolactin, parathyroid hormone.

The receptors foe estrogen, androgens, vitamin D3 are the receptors for osteoblastic nuclear steroid hormones.

1.13.4 OSTEOCLAST – BONE RESORPTION

The bone lining cell that is in charge of bone resorption is called an osteoclast. It is a massive multinucleated cell that can reach a diameter of up to 100 mm. It is derived from hematopoietic cells of the mononuclear lineage. Because of its inherent resorbtive action, it is typically located inside a lacuna (Howship's lacunae) and in touch with a calcified bone surface. Osteoclasts are rich in mitochondria, transport vesicles containing lysosomal enzymes, and Golgi complexes.

In the area of facing the bone matrix & the surrounding zone of attachment, they show more folding of the plasma membrane. The activity of osteoclast produces lysosomal enzymes for example tartrate-resistant acid phosphatase & cathepsin K that are released into the bone-resorbing compartment by its ruffled border.

The most important stage in the process of osteoclast attachment to the bone surface is its interaction with integrins, that are produced in osteoclasts that have a sequence of amino acid inside protein which is seen at the surface of bone matrix. After osteoclasts attached to bone matrix, then Avb3 integrin binding starts a cytoskeletal remodelling in the osteoclasts. The starting structure are mainly utilized for the attachment are known as podosomes.

The continuous construction & disassembly it initiates osteoclast migration with the surface of the bone that further promotes bone resorption. Many adhesion kinases that include protooncogene src, are needed for integrin signaling & the further production of podosomes. By acidifying & proteolyzing the bone matrix & hydroxy-patite crystals the are enclosed in the sealing zone, there the osteoclasts are able to resorbe the bone. The hydroxy-patite crystals are mobilized at the starting stage of bone matrix resorption when the bond with collagen is broken.

1.14 HOW SHOULD BONE HEALTH BE ASSESSED?

Dual energy X-ray absorptiometry (DEXA) is currently the most sensitive technique for determining bone mineral density (BMD) and, consequently, estimating the likelihood of future fractures (Elliott ME, Binkley N, 2004).

The hip and spine are the two key sites where DEXA measures bone mass. The acquired BMD values, which are expressed in grams per centimeter square, are compared to sizable databases made available by the DEXA equipment makers. T-scores and Z-scores are the two s.d. scores. While the Z-scores compare the BMD measures with an age-matched population, the T-scores compare the BMD values obtained with a sex-matched and race-matched population at peak BMD.

Osteopenia is defined by the WHO as patients with scores between 1 and 2.5 standard deviations below normal DEXA-values, and osteoporotic individuals with values greater than 2.5 standard deviations below normal (per WHO Study Group).

When assessing DEXA scans, a T-score of one is represented by 1 s.d. A patient with osteoporosis and an s.d. of 3 below normal will therefore have a T-score of -3. BMD measurements make it possible to identify high-risk patients before they break. Every standard deviation drop in BMD doubles the risk of fracture.

As of right now, there are no blood or urine markers that may be used to detect osteoporosis early on. However, serum tests for PTH, calcium, phosphorus, vitamin D metabolites, and markers of bone turnover, such as osteoblastic and osteoclastic functions, may reveal abnormalities in the metabolism of bone and mineral and indirectly suggest a higher risk of bone loss (Looker AC et al., 2000).

1.15 BONE REMODELLING

Bone mass and bone quality, the balance and rate of bone remodeling are essential. Local factors mediate the coupling between resorption and formation, and the RANK/RANK ligand/osteoprotegerin system is one of the major regulators. The activation of RANK, a receptor found on the cell membrane of both mature and precursor osteoclasts, promotes the differentiation and activity of osteoclasts. Osteoblasts or stromal cells release RANK ligand, which is the primary paracrine agent that initiates the bone remodelling unit. The RANK system in the osteoclast or osteoclast precursor needs to be activated by M-CSF. Osteoblasts also release OPG, a soluble decoy receptor that reduces osteoclast development and activity by neutralizing RANK ligand.

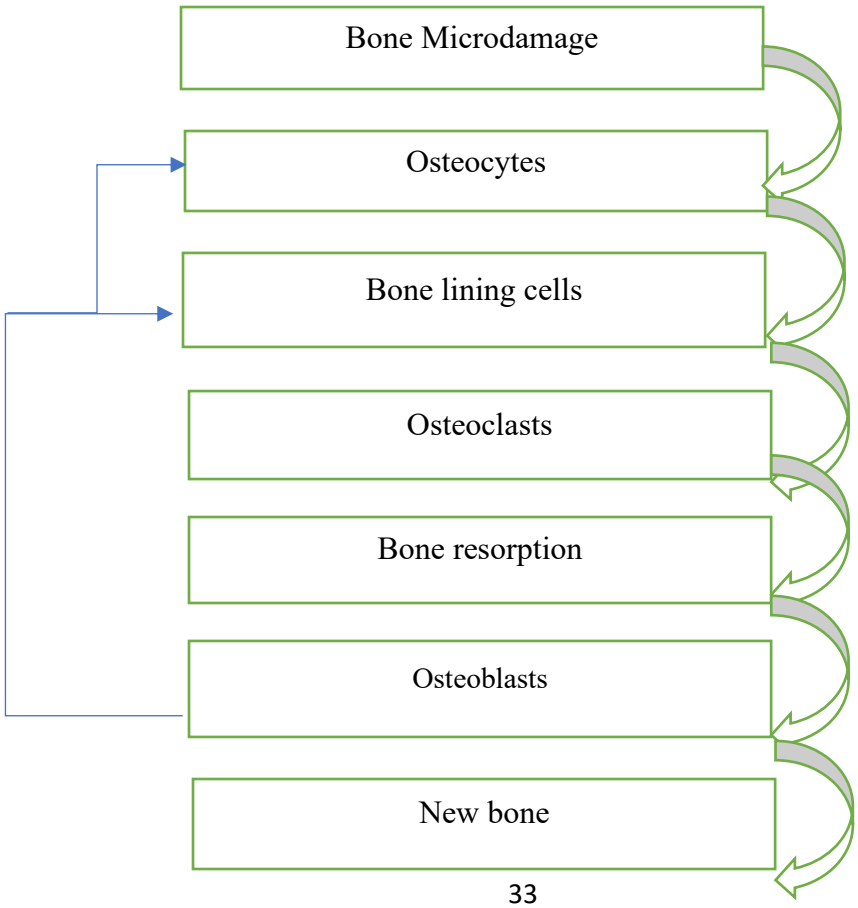

Fig. 1.7: Bone Remodelling and Healing

Hormones and cytokines, such as sex steroids, interleukin-1 (IL-1), and prostaglandin E2, control the release of RANK ligand and OPG. Numerous significant regulators of bone resorption may function by changing the proportions of RANK ligand and OPG that osteoblasts release.

There is very less knowledge known about the mechanisms that link bone production to resorption, ensuring that the resorption area is filled in. It is a locally regulated process, and substances secreted by osteoclasts and other nearby cells like macrophages, as well as factors released from the bone matrix during resorption, may play a role.

The osteoclasts are cells that break down bone in response to mechanical stress or microcracks in mineralized bone, which are detected by osteoclasts. Osteoclast function and development need the RANKL-RANK signalling pathway.

The molecule OPG prevents this route from functioning in live beings. By binding to the parathyroid receptor on osteoblasts, circulating PTH regulates blood calcium levels and indirectly stimulates osteoclast activity by increasing RANKL activity and decreasing OPG activity. Mature osteoclasts express the calcitonin receptor, which is activated by calcitonin.

However, the precise physiological effect of calcitonin beyond preventing bone breakdown by osteoclasts remains unclear. Estrogen improves bone health through influencing the activities of osteoblasts and osteoclasts. Resorbing osteoclasts secrete Cathepsin K over the ruffled border membrane, which is required for collagen breakdown. By releasing chemicals from the bone, osteoclasts attract osteoblasts to the site of bone resorption. The Wnt signalling pathway regulates osteoblast differentiation and function by interacting with Lipoprotein-related protein 5/6 and Frizzled co-receptors. Osteocytes produce endogenous inhibitors like sclerostin, which are more active when osteocytes are not under stress and regulate this pathway.

The three stages of the remodeling cycle are resorption, reversal, and creation. Partially developed mononuclear preosteoclasts migrate to the surface of the bone to form multinucleated osteoclasts, which is the first step in the resorption process.

After osteoclastic resorption ends, there occurs a reversal phase in which mononuclear cells appear on the surface of the bone. Once osteoclastic resorption has concluded, there is a reversal phase during which mononuclear cells appear on the surface of the bone. These cells provide signals for the migration and differentiation of osteoblasts and prepare the surface for the initiation of bone formation by newly generated osteoblasts. During the creation phase, which lasts until all of the resorbed bone has been replaced, osteoblasts add new bone.

As this stage is completed, the surface is further coated in flattened lining cells & a protracted period of resting takes place as it starts for a fresh remodelling cycle. The length of the remodelling cycles stage might change as up until the new bone structure is fully formed the resorption lasts for around 2 weeks, the reversal phase may take place upto 4 or 5 weeks and the formation can lead upto 4 months.

As it has been currently seen as what precise molecular mechanism shows the interaction in between the cells that belongs to osteoblastic & osteoclastic lineages. The osteoblastic lineage cells mediated the first activation of the bone remodelling cycle. The marrows osteocytes, lining cells and pre-osteoclasts may all be activated (Sudha T et al., 1999). Osteoprotegerin that is a secondary dimeric glycoprotein having molecular wt. 120 kDa which is a member of the TNF receptor family that further inhibits the actions of RANKL. The osteoblast lineage cells are the initial products of OPG, that is a soluble receptor which function as an antagonist & decoy receptor for RANKL.

As if there are other bone marrow cells that also makes up OPG. By the prevention of osteoclasts from reaching the final stage of differentiation & activation and also by triggering its apoptosis, OPG controls the resorption of bone. This OPG has purely reversible effects on bone resorption as is it not integrated into the bone matrix.

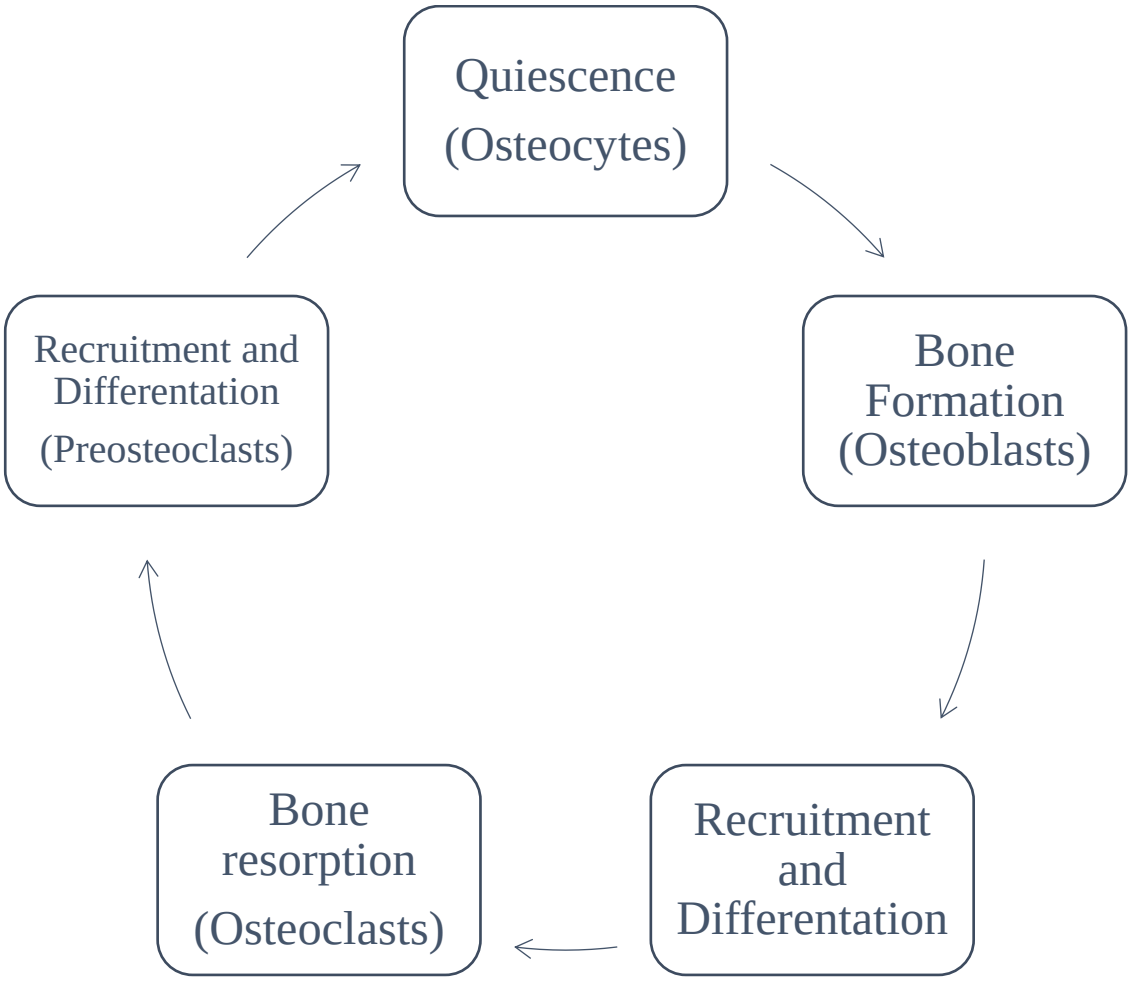

Fig. 1.8: Bone remodelling has five distinct faces

1.15.1 BONE REMODELLING: REGULATION

There are many substances along with hormones that are secreted by bone cells as well as bone hemopoietic bone marrow cells for regulating the proper integrity of bone. The function of bone cells is regulated through local as well as systemic process.

1.15.1.1 SYSTEMIC REGULATION

The most significant modulator of calcium homeostasis is parathyroid hormone. By promoting bone resorption, raising renal tubular calcium reabsorption, and enhancing renal calcitriol synthesis, it keeps serum calcium concentrations stable. When administered sporadically, PTH promotes bone growth; when released consistently, it promotes bone resorption (KIM CH et al., 2003). By improving intestinal absorption of calcium and phosphorus, calcitriol plays a crucial role in promoting bone mineralization. Furthermore, vitamin D3 has a twofold influence on bone turnover due to its significant anabolic effects on bone.

Through its receptor on osteoclasts, calcitonin, in pharmacologic quantities, mediates loss of the ruffled border, stops osteoclast motility, and inhibits the production of proteolytic enzymes. However, the physiologic role of this action in the adult skeleton is negligible and it is dosage limited. IGF-2 and the growth hormone (GH)/IGF-1 system is critical for skeletal growth, particularly at the cartilaginous end plates and during the production of endochondral bone.

Because of their impact on the control of bone resorption as well as creation, they are one of the main factors influencing adult bone mass. On bone cells, glucocorticoids have both stimulatory and inhibitory actions. By encouraging osteoblast differentiation from mesenchymal progenitors, they are crucial for osteoblast maturation; yet, they also reduce osteoblast activity (WANG J et al., 2004). Moreover, glucocorticoids increase osteoclast recruitment and make bone cells more sensitive to regulators of bone remodeling. Both bone growth and resorption are accelerated by thyroid hormones. As a result, hyperthyroidism increases bone turnover, which increases the risk of bone loss. Estrogens limit the production of osteoclasts by reducing the progenitor cells' reactivity to RANKL.

Moreover, estrogens promote osteoblast growth and inhibit apoptosis in addition to shortening the life span of osteoclasts. They alter the transcription factors, hormone receptors, enzyme, bone matrix protein, and IGF I, II, and TGF- genes. They also increase the local synthesis of OPG, IGF I, and TGF. Because androgens act on the androgen receptor, which is found in all types of bone cells, they are crucial for the growth and maintenance of the skeleton (Sato T et al., 2002).

1.15.1.2 LOCAL REGULATION

With the recent identification of the OPG/RANKL/RANK system, the overall management of osteoclastogenesis and bone remodeling is now better understood with relation to the local regulation of bone cell activity. Osteoclastic precursor cells express RANKL, which binds to RANK on the latter's surface. This interaction is essential for the differentiation, fusion into multinucleated cells, activation, and survival of osteoclastic cells.

By counteracting the effects of RANKL, OPG suppresses the system as a whole. Since it is the main agent influencing the pool of these precursor cells, macrophage colony-stimulating factor (M-CSF), which binds to its receptor, c-Fms, on preosteoclastic cells, appears to be essential for osteoclast development (UDAGAWA N et al., 1990).

The hypothesis that OPG and RANKL can be the mediators for the stimulatory or inhibitory effects of a variety of systemic hormones, growth factors, and cytokines on osteoclastogenesis is based on the opposite phenotypes of OPG-deficient or with RANKL overexpression (osteoporosis) and OPG-overexpression or with RANKL deletion mice (osteopetrosis).

The activity of the resorptive and antiresorptive agents "converges" at the level of these two mediators, whose ultimate ratio regulates the degree of osteoclast differentiation, activation, and apoptosis. This has been dubbed "the convergence hypothesis" in recent times (HOFBAUER, L.C et al., 2000).
This system is predominantly modulated by a multitude of cytokines, including TNF- and IL-10, which increase M-CSF synthesis and RANKL expression directly. Furthermore, a variety of other hormones and cytokines influence osteoclastogenesis by controlling the synthesis of OPG and RANKL in cells.

Furthermore, it appears that IL-6, a pleiotropic cytokine released by stromal cells, osteoblasts, and osteoclasts, is a significant regulator of bone remodeling by both encouraging osteoblast formation in high bone turnover settings and boosting osteoclastic bone resorption. According to recent research, osteoblast-derived PTHrP is a crucial regulator of bone cell activity because it encourages the recruitment of osteogenic cells and inhibits osteoblasts' apoptotic death.

A multitude of skeletal illnesses can result from abnormalities in bone remodeling. New methods for the detection and treatment of skeletal illnesses have been made possible by recent developments in the understanding of the local and systemic regulation of bone remodeling. More recently, advances in molecular and cellular biology have made it possible to identify the aberrations in osteoblastic and osteoclastic lineage cells that cause bone disease and to design novel therapeutic strategies based on a deeper comprehension of the pathogenetic mechanisms.

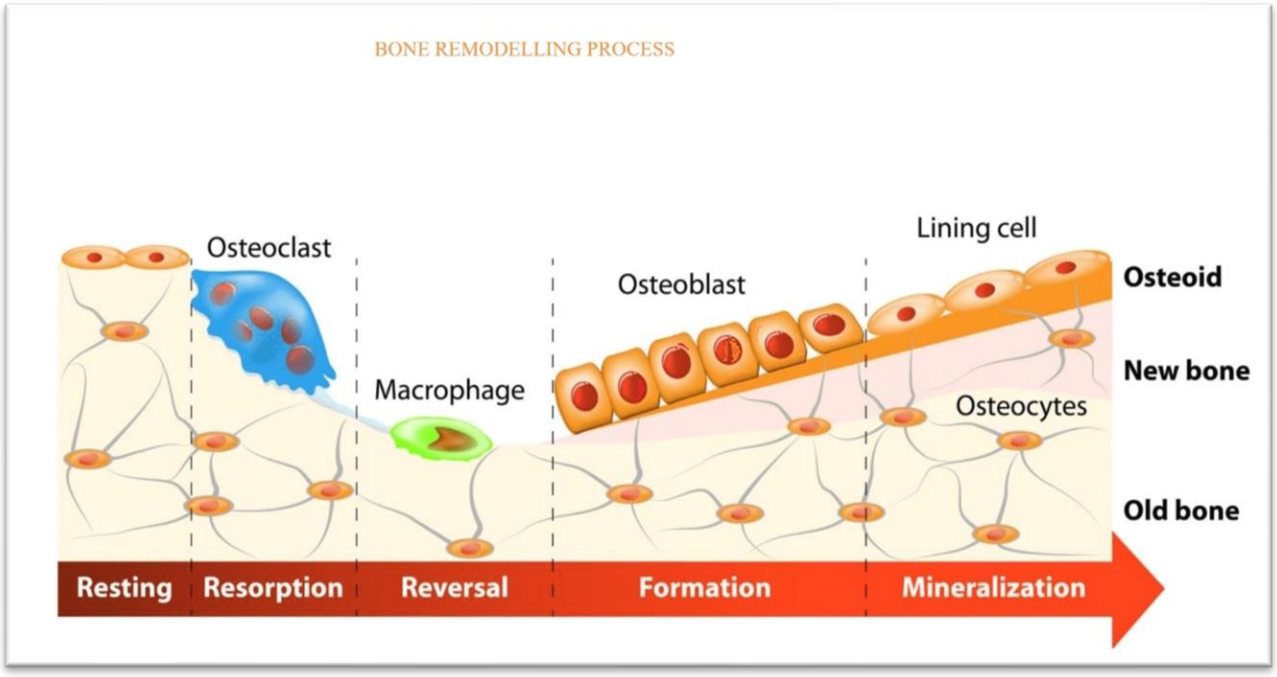

Fig. 1.9: Bone Remodelling Process

1.16 BONE TURNOVER INDICATORS

There is no evidence that the number of indicators of bone production & resorption can be added for determine the remodelling imbalance. The bio-chemical markers of bone turnover explain a great degree of increase in the full bone turnover. The measurement of enzymatic activity of the osteoblast cells or the osteoclast cells in the form of alkaline & acid phosphatase activity or by measuring the components of the bone matrix that is released into the circulation during the time of formation or resorption can help in determination of the rates of bone formation.

There are different types of bone turnovers that can be further divided as Bone resorption markers, Markers the shows osteoclast number, Bone formation markers.

Osteoclast-mediated bone resorption results in the release of indicators for bone resorption from the bone matrix. The osteoclasts secrete cathepsin K at the time of bone resorption that causes more proteolysis & releases collagen fragments form the bone. The two majority bone resorption markers are C-terminal cross linked telopeptide of type-I collagen fragments & N-terminal telopeptide of type-I collagen fragments (Leeming DJ et al., 2006).

Bone turnover markers may be helpful in tracking the effectiveness of treatment, particularly antiresorptive medication, for osteoporosis. They have been shown to predict the pace of postmenopausal bone loss and the incidence of osteoporotic fractures.

The two facets of osteoblast function—differentiation and bone formation—are linked to the indicators of bone formation. Procollagen fragments generated during collagen integration into the newly created bone matrix are the markers directly reflecting bone formation, whereas molecules specific to bone, such as osteocalcin and bone-specific alkaline phosphatase, are the markers showing differentiation. These include the procollagen type I N-terminal propeptide and procollagen type I C-terminal propeptide (Vasikaran et al.,2011)

Markers	Tissue of Origin	Analytical Method	Specificity
Total Alkaline Phosphatase (Total ALP)	Bone, liver, kidney, intestine, placenta	Colorimetric	Mainly for bone formation and only in the absence of liver
Bone Specific Alkaline Phosphatase (b ALP)	Bone	Colorimetric	Specified products of osteoblasts, few assays show upto 20% cross-reactivity along with liver iso-enzymes
Osteocalcin (OC)	Bone, Platelets,	RIA, ELISA, CLIA	They are specified products of osteoblasts, immunoreactive forms of blood
Procollagen type I carboxy-terminal propeptide (PCP)	Bone, skin	RIA, ELISA	Specific product of proliferating osteoblasts and fibroblasts
Procollagen type I amino – terminal propeptide (PENP)	Bone, skin	RIA, ELISA	Specific products of proliferating osteoblasts and fibroblasts

Table 1.1: The Different markers with specificity for bone formation.

1.16.1 INDICATORS OF BONE LOSS

Except for tartrate-resistant acid phosphatase, most of the markers of bone resorption are made from bone collagen that has broken down. Bone sialoprotein and osteopontin, which are not made of collagen, have only recently been looked at as markers of bone turnover. Until recently, most

bone resorption marker tests were only done on urine. However, newer tests can now also be done on serum or plasma (Seibel, 2000). Below, you can see the molecular signs of bone resorption.

1.17 NEW BONE PROTECTIVE TARGETS AND STRATEGIES

For the treatment of osteoporosis, researchers are looking into a number of new targets. Here are the most recent new targets and the drugs used to treat them.

1.17.1 RANKL

RANKL is a homo-trimeric protein that is either released by some cells as activated T-cells or by membrane bound on osteoblastic & activated T cells. Through either of the proteolytic cleavages the protein released is produced from its membrane form. The proteolytic cleavage of RANKL is carried out by matrix metalloproteases or ADAM (A. Hikita et al., 2006).

The majority of known stimulants of osteoclast activity and development cause osteoblastic stromal cells to produce RANKL. But RANKL is also expressed at modest levels in a number of other organs, such as the spleen and bone marrow, and at high levels in lymph nodes, the thymus, mammary glands, and the lung. It is expressed in the joints of inflammatory arthritis patients by synovial cells and activated T cells, which may play a role in the joint degradation observed in rheumatoid arthritis patients.

Denosumab prevents osteoclasts from differentiating, becoming active, and surviving by binding to RANKL and blocking the interaction between RANKL and RANK. As a result, bone resorption is slowed.

1.17.2 CATHEPSIN K INHIBITORS

The tight osteoclastic adherence at the bone surface is necessary to close-off and extracellular compartment that is known as resorption lacunae & starts the bone resorption cycle. In order for producing an acidic environment for dissolution of bone minerals, osteoclast leak these proteins into resorption lacunae. Proteases that break down collagenous and non-collagenous bone matrix proteins are then released. CatK is one of the several collagenases that belong to the papain family of lysosomal cysteine proteases. It is highly expressed in osteoclasts and has the ability to cleave the telopeptide and helical sections of type-collagen (Duong LT., 2012). Osteoclasts and other multinucleated cells such as Langhans cells and large foreign body cells exhibit a high level of it.

1.17.3 SCLEROSTIN

Sclerosteosis & van-Buchem disease are the two rarest recessive autosomal, sclerosing bone disorder that is characterized by high bone mass & more bone strength that is caused by defects in the SOST gene in chromosome 17q12-21 that further encodes sclerostin (Lots GG et al., 2005).

At the time of inactivating mutations of SOST that induces sclerosteosis, van Buchem disease which is caused by 52 kb homozygous non-coding deletion that is placed on 35 kb downstream of SOST gene and contains a regulatory region of SOST transcription. Sclerostin that is secreted by matured osteocytes placed in mineralized matrix by inhibiting bone formation at the surface of bone through binding to LRP5/6 co-receptors and antagonizing canonical, beta-catenin dependent, Wnt signaling in osteoblasts (Li X et al., 2005). Sclerostin enhance the reabsorption of osteoclastic bone through acting on nearby osteocytes and by raising RANKL expression and RANKL/OPG ratio. This shows a catabolic effect on bone along with its detrimental effects on bone growth.

1.17.4 DICKKOPF-1 ANTIBODY

Dickkopf-1 (Dkk1) is a soluble inhibitor of β-catenin/Wingless type signaling, which is necessary for the development of tissues that induce the formation of an embryonic head in frog embryos. According to Mac Donald et al. (2007), Dkk1 is a negative regulator of proper bone homeostasis in vivo and is linked to the regulation of osteoblast differentiation. Dkk1 activation in osteoblasts appears to be involved in the pathophysiology of glucocorticoid- and estrogen deficiency-mediated osteoporosis, whereas Dkk1 overexpression in osteoblasts promotes osteopenia and slows fracture repair.

According to a publication, there exists a negative correlation between serum DKK1 levels and dual-energy x-ray absorptiometry (DXA) bone mineral density (BMD) at the lumbar spine and femur in people. Overall, it is unclear how aging and BMD affect the expression of sclerostin and DKK1, and more research is needed to clarify this.

1.17.5 SEROTONIN

Scientists have recently discovered that serotonin plays a crucial role in the process by which osteoblasts lay down bone. It seems to have various effects on the body depending on whether it is produced in the gut or the brain. Drugs that limit the production of serotonin in the intestines have been found to promote bone formation, halt bone loss, and increase bone mass in proof-of-principle research using ovariectomized mice (Yadav et al., 2010). Osteoporosis treatment may soon include medications that inhibit serotonin production in the intestines.

1.17.6 CALCITOLYTIC AGENTS

Calcilytics are a new type of bone-building drug. They block the calcium sensing receptors and make hypocalcemia look like it is happening. This causes a short pulse of PTH secretion. In contrast to PTH therapy, calcilytics are taken by mouth and do not require injections. The fact that calcilytics have a narrow therapeutic index has been one of their biggest problems in real life. In theory, a high-amplitude PTH pulse followed by a quick return to normal means that bones grow faster. These compounds caused PTH to keep being released, and the results were similar to what is seen in a disease called primary hyperparathyroidism, which breaks down bone.

1.18 MAIN DRUG PROFILE: RALOXIFENE

There is one benzothiophene-specific estrogen receptor modulator (SERM) Raloxifene. Despite not being a hormone, raloxifene appears to bind to estrogen receptors in order to mediate its biological effects.

Certain tissues experience the activation of estrogenic pathways whereas other tissues experience the blockage of estrogenic pathways as a result of this binding, which causes conformational changes in the estrogen receptor that are different from those brought on by estrogen1–5. In bone, raloxifene seems to function as an estrogen agonist. It raises bone mineral density (BMD), lowers the frequency of fractures, and reduces bone resorption and turnover. (Delmas PD et al., 2002).

1.18.1 WORKING

Most of what raloxifene does in the body is done by binding to oestrogen receptors. Because of this binding, some estrogenic pathways are turned on and others are shut down. So, raloxifene is a selective oestrogen receptor modulator and an oestrogen agonist/antagonist (SERM).

Raloxifene occupies the same receptor ligand-binding site as estradiol. Oestrogen response elements are short, palindromic sequences of DNA that are recognized by homodimers. This results in a structural alteration of the receptor's C-terminal alpha helix, which then prevents activation function-2 from being accessed. Because of this, it's likely that it's difficult to gain access to the transcriptional coactivators that are required to assist turn on the oestrogen-responsive genes.

1.18.2 PHARMACOKINETICS

After being quickly absorbed from the digestive system, raloxifene experiences significant first pass glucuronidation. Although over 60% of an oral dosage is absorbed, absolute bioavailability is just 2% due to significant pre-systemic glucuronide conjugation. Changes in enterohepatic recycling and glucuronide production rates may lead to significant interpatient variations in bioavailability.

It is anticipated that if the suggested 60 mg dose is administered, the mean peak Cmax will be 0.5 ng/mL.16 It is anticipated that several doses of 60 mg will result in a mean Cmax of 1.36 ng/mL.

Raloxifene is extensively dispersed throughout tissues; following an oral dose of 30–150 mg, the volume of distribution (V) is 2348 L/kg. V does not rely on dose. It appears that the medication is converted to an active metabolite in a number of tissues, including the kidneys, lungs, spleen, liver, and uterus. In vitro, albumin and α1-acid glycoprotein are 95% bound to raloxifene and its conjugates. There is a significant first-pass metabolism of raloxifene. Raloxifene 4′-glucuronide, 6-glucuronide, and 6,4′-diglucuronide are examples of conjugate formation.

There is very little Raloxifene that is found free. The main form of excretion for raloxifene is feces. After being removed from the body in the biliary tract, glucose metabolites are converted back into the original medication by bacteria. In the urine, less than 0.2% of raloxifene is eliminated unaltered, and less than 6% is eliminated as glucuronide conjugates. (Knadler MP et al., 1995).

1.18.3 HOW AND WHY, IT'S USED:

Raloxifene is used to treat or prevent osteoporosis and lower the risk of invasive breast cancer in postmenopausal women (PMW) who have osteoporosis or a high risk of developing breast cancer. Raloxifene will help most women by lowering their risk of both invasive breast cancer and bone fractures. It is not a first-line treatment for cardiovascular disease and cholesterol problems, even with a strong profile for these conditions.

1.18.4 CONTRAINDICATIONS

Raloxifene shouldn't be given to men, women who could have children, or people who have or have had venous thromboembolism, which includes deep vein thrombosis, pulmonary embolism, and retinal vein thrombosis. It is also not a good idea for people who have problems with their liver, like cholestasis. Other reasons not to take it are an allergy to raloxifene, severe kidney disease, or bleeding in the uterus that can't be explained. It shouldn't be given to people with breast or endometrial cancer.

1.18.5 EFFECTS THAT ARE BAD

Hot flashes (vasodilation), idiopathic leg cramps, venous thromboembolism (deep vein thrombosis, pulmonary embolism, and retinal vein thrombosis), and venous thromboembolism are all possible side effects. The number of platelets dropped by 6-10% and various liver enzymes (AST and/or ALT) rose moderately, according to reports.

1.19 MECHANISMS OF BONE LOSS WITH ANTIEPILEPTIC DRUGS

One typical explanation for bone loss in epileptic patients is a deficiency in vitamin D, which is necessary for bone remodeling and growth.[13] It has been discovered that stimulating the hepatic CYP450 system speeds up the breakdown of vitamin D into its polar inactive metabolites, reducing the amount of vitamin D that is physiologically active. Lower blood 25(OH)D concentrations are observed in both adults and children (Krishnamoorthy G et al., 2009).

Some studies have demonstrated signs of accelerated bone turnover even in the absence of Vitamin D insufficiency, and not all research assessing the effect of AED on bone health have consistently found Vitamin D deficit. Being an enzyme inhibitor, valproic acid is also linked to a drop in bone mineral density and an elevated risk of fractures (Lee R et al., 2012).

Consequently, it has been proposed that AEDs may impact bone metabolism by pathways other than hepatic enzyme activation. Reduced dietary calcium absorption is linked to a drop in physiologically active forms of vitamin D, which causes hypocalcemia and feedback hypersecretion of circulating PTH. Increased bone resorption and subsequent BMD reduction and fracture risk are the results of hyperparathyroidism.

Another important factor that is believed to be crucial is inhibition of the cellular response to PTH. AEDs' direct effects on bone cells, their direct inhibition of the intestine's absorption of calcium, their reduction of osteoblast cell proliferation, and their inhibition of calcitonin release are additional pathways (Fitzpatrick LA., 2004).

It has been demonstrated that enzyme-inducing AEDs, such DPH and CBZ, lower vitamin D levels. Vitamin D deficiency can cause hypophosphatemia, hypocalcemia, and secondary hyperparathyroidism, which can all result in bone loss. Numerous studies have linked the use of AEDs to low levels of vitamin D, however no discernible correlation has been found between vitamin D levels and BMD.

In addition to secondary hyperparathyroidism and calcium homeostasis, vitamin D has important additional effects. It promotes differentiation along the osteoclastic lines and is a significant regulator of osteoblastic function. Pregnane X receptor, also known as the orphan nuclear receptor, is most likely the pathway by which vitamin D insufficiency linked to AED usage is mediated (PXR) (Pascussi JM et al., 2005).

The PXR is expressed in the kidney, liver, and intestine. Its DNA binding domains are 60% similar to those of the vitamin D receptors (VDRs). It has been demonstrated that PXR mediates the induction of the cytochrome P450 enzymes involved in drug metabolism, CYP 2 and CYP 3.

Adult bone mass is made up of two primary forms of bone: trabecular, which makes up 20% of the mass, and cortical, which makes up about 80%. Cortical bone preserves the mechanical strength and integrity of the bone and is dense, with a turnover rate of only 3% annually. On the other hand, trabecular bone, which is present in long bones and vertebrae, is more

metabolically active, responsive to hormonal stimuli, and has a lower mineralized content. It also turnovers at a rate of about 26% annually.

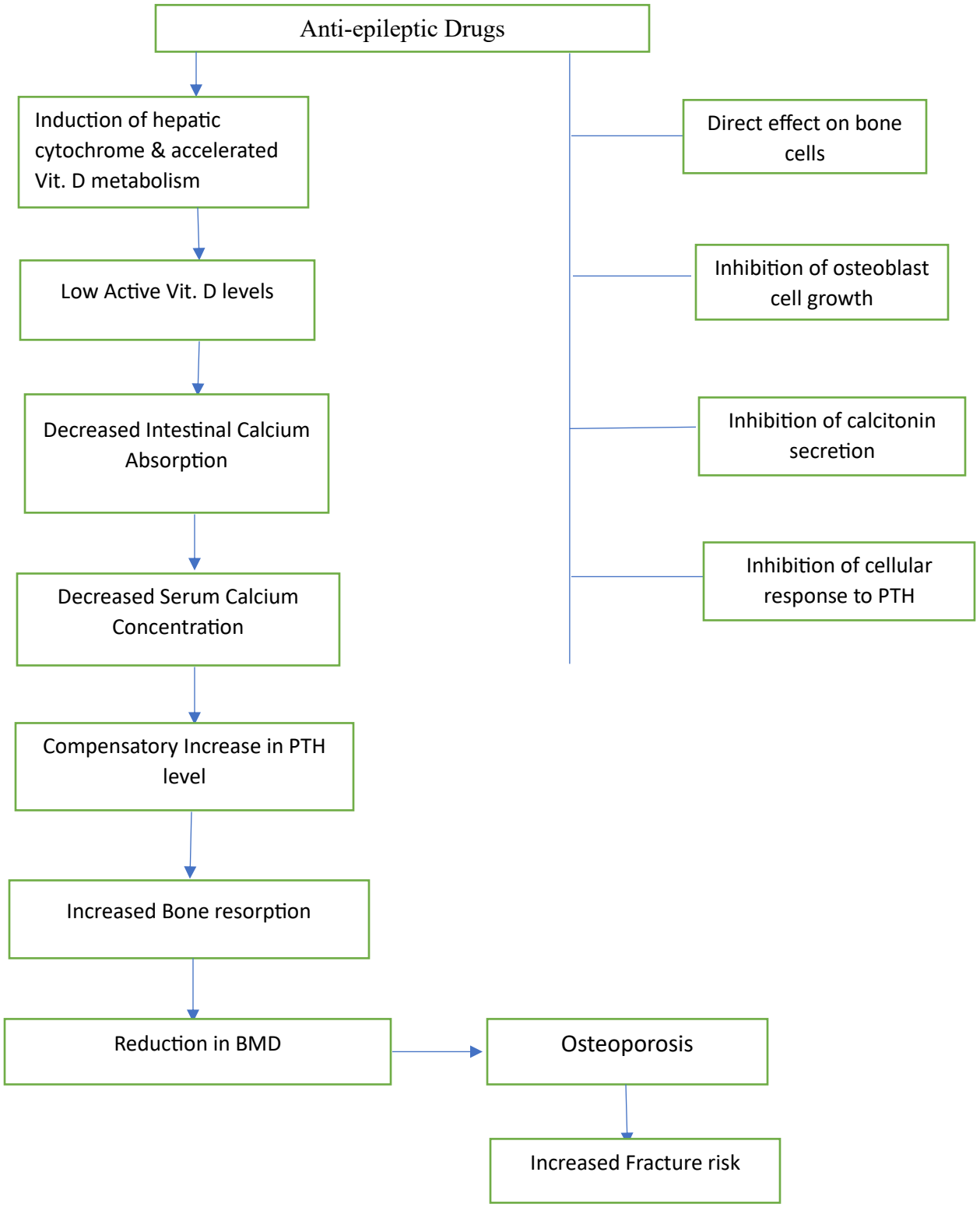

Figure 1.10: Proposed mechanism to anti-epileptic drug induced degenerative disease.

1.20 EXPERIMENTAL MODELS OF OSTEOPOROSIS

Biology and medicine both rely heavily on animal models in experimental investigations to examine the variables that alter bone turnover and structure. There is a large form of animal-based osteoporosis modelling that allows the exploration of specified charactertics of disorders that affects different bones, length of its pathological process, manifestations in its compact, trabecular bone, body changes. Osteoporosis is a multifunctional metabolic disorder whose pathogenesis takes a site of endogenous as well as exogenous factors that affects the bone tissue.

One can describe the remodeling of the bone based on histological, morphometric, and biochemical studies using different experimental osteoporosis models. Biomechanical methods can also be used to test the quality and strength of the bone under different osteoporosis triggers and to identify some pathogenic pathways of this condition.

In order to investigate the impact of bone quality on biomaterial restructure and construction stability, it is also possible to test different fixative constructions and biomaterials implanted in the bone using animals that have been given a model of osteoporosis. The osteoporosis model, which is based on animals with traumatic bone injuries, aids in assessing the parameters of reparative osteogenesis related to this condition and identifying the risk factors that lead to nonunion of the bones.

The aforementioned elements demonstrate the relevance and significance of using experimental models to replicate a disease as widespread as osteoporosis. Numerous animals, including dogs, ewes, primates, rabbits, mice, and rats, are used as models for osteoporosis. However, the majority of experimental procedures use laboratory rats and mice since these studies.

First and foremost, the experimental replication of this pathology makes it possible to identify a specific pathogenic pathway. Secondly, the researcher can study the molecular, cellular, and systemic changes using the experimental model, which is not possible with a patient's clinical examination. Lastly, the experimental model of the pathological condition is an important object of therapeutic efficacy evaluation for this type of pathology.

Any study's animal selection process should be guided by the following guidelines:

1) applicability as an analog;
2) informativeness; and
3) genetic regularity of the employed organisms;
4)a basic understanding of biological characteristics;
5) accessibility and cost-effectiveness;
6) findings summary;
7) practicality and convenience of use for the experimental manipulations;
8) environmental factors;
9) moral and societal ramifications

One can use a variety of techniques to imitate both primary and secondary osteoporosis. Type 1 osteoporosis, or postmenopausal osteoporosis, is brought on by ovariectomy. Secondary osteoporosis can also be mimicked by means of orchiectomy, thyroidectomy, tenotomy, sciatic nerve or spinal cord injury, limb amputation, lack of skeletal loading or zero gravity, chemicals, etc.

1.21 METHODS OF BONE ASSESSMENT UNDER OSTEOPOROSIS MODELLING

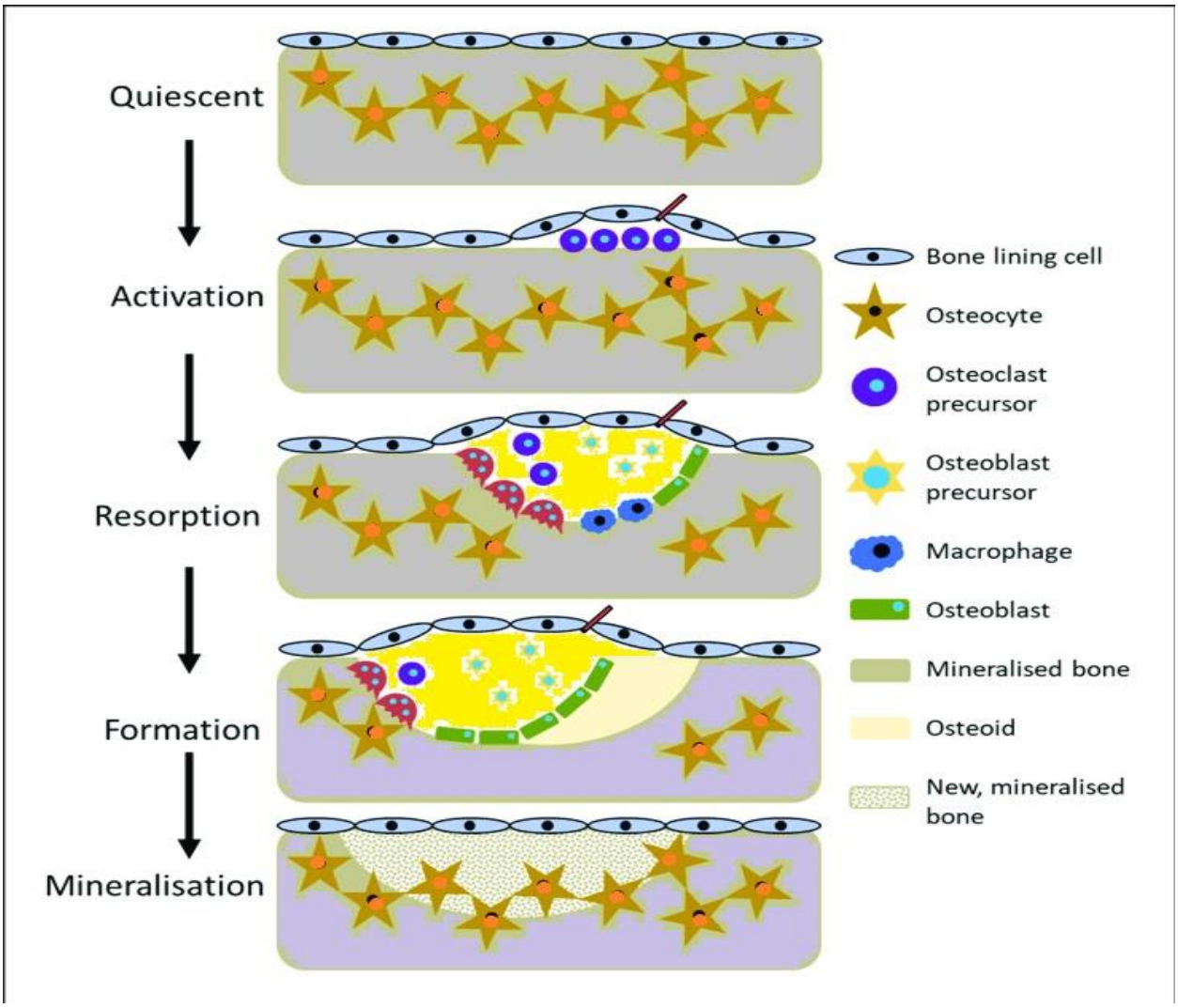

Fig. 1.12: Process of bone under Osteoporosis modelling

The overall mass of the bone, the geometry of the bone, and the characteristics of the constituent tissue are some of the elements that affect a complete bone's structural integrity. Even though there are many factors that affect bone strength, bone mass—which is determined by bone mineral density, or BMD—is the main factor utilized in clinical settings to diagnose osteoporosis and gauge fracture risk. Dual-energy x-ray absorptiometry is used to measure bone mass. Clinical and

scientific interest in alternative measures of bone quality that may enhance fracture risk prediction has increased due to BMD's limitations as a fracture risk predictor.

Mechanical testing enables the characterisation of various structural and material qualities by providing a direct evaluation of a variety of mechanical parameters over a variety of length scales. Whole-bone testing enables evaluation of the macroscopic structural characteristics of bones, such as strength and structural stiffness. Material testing procedures allow the assessment of intrinsic tissue qualities like ultimate stress and elastic modulus at smaller length scales.

Whole-bone mechanical testing is used to evaluate the structural behavior of bones at the macroscale. Usually, a whole bone is loaded to failure in torsion, bending, or compression during these tests. The structural stiffness, the failure load, and the energy required to fail are examples of outcomes. The ability of the bone to withstand elastic, or reversible, deformation is reflected in its structural stiffness. The strength of the bone is characterized by the failure load. The amount of energy a bone can withstand before breaking is known as its structural toughness, and it is measured by the energy absorbed to failure. Destructive whole-bone testing is necessary for the experimental evaluation of bone strength, and testing to failure has the intrinsic limitation of breaking the specimen.

The mechanical characteristics of cortical and cancellous tissue are evaluated through mechanical testing of bulk tissue specimens removed from whole bones. This kind of testing has been used to describe how various factors, such as tissue mineral content, porosity, apparent density, and anatomic site, affect the mechanical characteristics of bone tissue.

The methods in separating material charactertics of bone tissue that have been prepared with the use of microbeam specimens. This test takes applying bending loads to the microbeam that is made up of trabecular & cortical bone that measures around 200 9 200 9 2000 lm. The elastic module and its stress are seen in the results. The inherent stress of the materials to its plastic deformation is seen by its yield stress. The material properties that are taken from its experiments as incorporated by impact of discontinuities but it came to know that they are not dependent on the macroscopic bone geometry & micro-architecture of trabecular.

As an alternative, bone tissue's material characteristics can be assessed at the meso- to microscale using traditional indentation testing. An indentation test involves pressing a rigid indenter into a flat object with a known force. The area of the resulting impression is then assessed optically. The hardness of a substance is determined by dividing its force by the area of the imprint. This value indicates the material's resistance to plastic deformation. Characterizing the mechanical characteristics of individual trabeculae or osteons is made possible by microindentation. Benefits include the capacity to measure in many areas inside the tissue and the comparatively simple nature of testing. The fact that this method only produces tissue hardness is a disadvantage.

Nanoindentation is able to probe the mechanical characteristics of small volumes of tissue, down to the level of individual lamellae, at the microscale. This method uses an indentation test with a depth-sensing indenter tip, frequently in conjunction with a scanning probe microscope to provide measurements that are spatially resolved. In order to determine the hardness and indentation modulus, the force-displacement data are evaluated. In bone tissue, nanoindentation at

comparatively shallow indentation depths of about 100 nm produces spatial resolutions of about 1 lm. The capacity to quantify the material characteristics of microstructural elements like lamellae and identify localized alterations in bone material properties brought on by illness or medication therapy are two benefits of this technology.

Evaluation of three-dimensional (3D) bone macroscopically Quantitative CT allows for the in vivo performance of geometry. (QCT). In QCT, an object of interest attenuates the x-rays produced by the x-ray source, and the signal is detected by a detector on the other side. A three-dimensional picture of x-ray attenuation is created using tomographic methods as the source and detector revolve around the item. The 3D macroscopic bone geometry obtained from QCT shows discrete cortical and trabecular bone, as well as apparent volumetric bone mass density (v BMD, or mass mineral/total volume [bone + marrow]). This method's strength is its ability to photograph vertebral locations, despite its in-plane resolution (about 0.5 mm) is not good enough to distinguish trabecular architecture.

The development of high-resolution peripheral QCT (HR-pQCT) scanners has made it possible to image 3D trabecular morphology in vivo at peripheral locations such as the distal radius. These scanners have an isotropic resolution of about 80 lm. This method's main benefit is that it allows for the resolution of trabecular bone and the calculation of morphologic parameters such bone volume fraction (BV/TV), trabecular thickness (Tb. Th), trabecular separation (Tb. Sp), and trabecular number (Tb. N). It is also feasible to compute apparent vBMD by incorporating calibration phantoms. Partial volume effects influence the morphologic parameters since the spatial resolution is close to the size of trabeculae; yet, the HR-pQCT trabecular measures are correlated with those measured by micro-CT, the gold standard currently utilized for assessing trabecular morphology.

Peripheral trabecular network nonionizing 3D imaging is made possible by high-resolution MRI (HR-MRI). In order to create three-dimensional (3D) photographs of the hydrogen in the water within the skeletal tissues, a high magnetic field and a series of radiofrequency (RF) pulses are applied to the specimen during scanning. Because of its low water content and the protons' chemical environment within the bone matrix, bone tissue does not produce any signal in normal magnetic resonance imaging. Instead, the trabeculae show up as the black area inside the bright marrow during imaging. Resolutions of 156 9 156 9300 lm are normal in vivo, and as tiny as about 50 9 50 9 200 lm have been attained ex vivo. Consequently, trabecular morphologic analysis based on MRI

This range is found to be more in animals, the process used in evaluation of bone mass, architecture & turnover in animals with simulated osteopenia & osteoporosis are same to that which are used foe the same purpose in humans. The rates of biochemical markers of calcium, phosphorus & magnesium in blood & urine along with major proteins secreted by osteoblasts and osteoclasts as the time of bone remodelling are measured in both humans as well as animals. The markers for bone formation are N-or C- telopeptide of Type 1 collagen & tartrate -resistant acid phosphatase.

Animals with modeled osteoporosis can have their BMD measured with the aid of contemporary bone densitometers. Dual-energy X-ray absorptiometry (DXA) is widely used to measure the bone mineral content (BMC) and bone mineral density (BMD) of an animal's entire skeleton.

Mice and rats are two small creatures that can use it. Compared to DXA, peripheral quantitative computer tomography (pQCT) offers the benefit of allowing for the independent analysis of trabecular and cortical bones. While both pQCT and DXA offer valuable insights into bone mineral density and fracture risk, the significance of histology and morphometry is comparable.

In order to broaden our understanding of bone remodeling under osteoporosis caused by various variables, they are evaluating compact and trabecular bone architecture, trabecular volume, osteoblast, osteoclast, and osteocyte populations, as well as defining dynamic parameters of bone production. The biomechanical investigations are carried out using experimental animal models. The long bones are examined by twisting tests and bending them in three to four places in order to evaluate the mechanical qualities. The vertebrae and femoral neck can be evaluated using the compression test or a bending-and-compression test combination.
In order to better understand osteoporosis, many animal models have been developed. Both naturally existing and experimentally altered animal species exhibit osteoporosis-like characteristics. Dietary changes, surgical procedures, and other factors can potentially contribute to osteoporosis and osteopenia. However, only a small number of models have accurately represented the complete spectrum of illness symptoms, including the fact that bones spontaneously shatter.

Osteoporosis can develop for no apparent reason or as a secondary effect of another disease or condition. Further, primary osteoporosis can be classified into two distinct forms: type I and type II. Vertebral fractures are more likely to occur in people with Type I osteoporosis, commonly known as postmenopausal osteoporosis, due to increased bone turnover and rapid cancellous bone loss. Both older women and men are susceptible to developing type II osteoporosis, often known as senile or age-related osteoporosis. The root of this disease is less obvious than that of type I osteoporosis.

1.22 SPONTANEOUS ANIMAL MODELS OF OSTEOPOROSIS

The bone mass decreases with the age in both the cases inbred as well as outbred mice. The outbred CW-1 has lost upto 60 % of its trabecular & cortical bone tissue at the time of its birth. As the age of in-between 6 & 24 months, the trabecular bone volume in the proximal tibia reduces by 60% and the cortical bone thickness in the tibia diaphysis reduces by 21% in inbreed mice such as C57BL/6J. On further basis it was evidenced that these strains of mice will be useful in studying age-related type-II osteoporosis. SAMP6 mice were the first one to be used as rodent model of senile osteoporosis have many fractures in its older age. It helped as an main model for studying osteoporosis & bone fragility.

1.22.1 CAUSED OSTEOPOROSIS IN ANIMAL MODELS

All experimental osteoporosis protocols can be implemented in skeletally immature or mature rats (Shen V et al.,1997). Rats' skeletons are deemed developed after 10 months, even though they

reach sexual maturity at 2.5 months. A low peak bone mass is attained in skeletally young rats, which is thought to be a high-risk factor for osteoporotic fractures in humans. Because of this characteristic, skeletally immature rats are a suitable model animal for studying endocrine, dietary, and environmental factors that can affect peak bone mass.

The lack of an easy-to-use animal model has made it harder to come up with treatments for osteoporosis. But most of the time, animal models of osteoporosis are used to test how drugs affect bones before they are used on people. Due to the fact that spontaneous animal models of osteoporosis show individual differences, like osteoarthritis models, many different induced models are used to directly cause osteoporosis in many different species. These include chemical injections, diet manipulation, immobilization, surgery, and other methods.

Choosing the appropriate osteoporosis model animal is crucial. Various animal species and human bone tissues used as osteoporosis models have distinct histological and biological metabolic features. Basic bone mass measurements vary between genders and ages, and there are rigorous guidelines when selecting an animal species to use as an osteoporosis model. Currently, rats, mice, dogs, lambs, rabbits, pigs, and primates are among the frequently utilized species. Our study results indicate that over 60% of the research have utilized rats and mice, with lambs and rabbits coming in second and third. Relatively few other non-human primates, such dogs and pigs, have also been employed.

The rat is the most commonly used laboratory animal in osteoporosis research, and it has evolved into the most popular and sophisticated model animal for the condition. Because they are readily available, affordable, grow quickly, have a short lifespan, and have good skeletal traits, rats are frequently used as model subjects. Humans and rodents share many characteristics, including the genome. Age, a similar distribution of cancellous bone, a high rate of bone conversion following ovariectomy, a reduction in intestinal calcium absorption, and a comparable reaction to sex hormones are all factors that contribute to bone loss.

Mice have been a popular experimental animal in the field of bone mass gene control due to the thorough understanding of the mouse genome. In order to investigate the genetic aspects of bone metabolism, mice are frequently employed. Target genes are either imported or knocked out, and phenotypic and pathological alterations are monitored. The main experimental model animals used in the investigation of the genetic variables that influence peak bone mass and age-related bone loss are mice. The sluggish epiphysis closure and shorter bone rebuilding cycle compared to people are the drawbacks, though, and they could skew the experiment's findings.

Adult rabbits exhibit clear Haversian system repair capabilities, a quicker rate of bone turnover (16), and an earlier epiphyseal closure period (typically 6–8 months) than rodents. Rabbits have been employed more frequently for ovariectomized, glucocorticoid, and ovariectomized + glucocorticoid osteoporosis animal modeling, according to the results of the included literature. A Haversian reconstruction system is also present in sheep, pigs, dogs, and non-human primates; these species share oestrus cycles and are genetically closer to humans than humans. They are not utilized in osteoporosis models because they are costly, difficult to manage, and hormone alterations have little effect on bone loss.

The majority of studies have assessed the bone mineral content (BMC) and bone mineral density (BMD) of the lumbar spine, femur, and tibia because these are the most often occurring clinical fracture sites. Using a dual-energy X-ray (DXA) scanner is the primary way of measurement. Then, bone biomechanics indexes, blood sample biochemical parameters (such as calcium and phosphorus), and bone histometrics were established. When comparing different signs, the estradiol can be elevated for postmenopausal osteoporosis because this type of osteoporosis is associated with a decrease in oestrogen. The aforementioned indications can all represent the state of osteoporosis in animals in various ways. To reach a full conclusion on modeling effects, a thorough analysis of a range of indicators is required.

1.23 RALOXIFENE AS A POSSIBLE TREATMENT FOR AED INDUCED OSTEOPOROSIS

Raloxifene is an antiestrogen in endometrial and breast tissue and a selective modulator of estrogen receptors that partially replicates the effects of estrogens in bone and the cardiovascular system. RLX works similarly to estrogen to stop osteoclastic activity and bone remodeling because it has a high affinity for the estrogen receptor (Bryant, 2001).

It has the same effect as bisphosphonates when reducing the risk of vertebral fractures. Postmenopausal osteoporosis is the most common type of fracture in women. RLX has been shown to help prevent morphometric and clinical vertebral fractures, which are the most common type of fracture. The lumbar spine BMD is also linked to an early rise. It also improves biochemical markers of bone turnover and a person's cholesterol level. Besides being good for bones, it also fights against estrogen (Delmas et al., 1997). For this reason, the FDA approved RLX for postmenopausal women who were at a high risk of getting breast cancer in 2007.

Some medicines have been approved to help people with degenerative diseases. Estrogen successor, selective estrogen receptor modulators (SERMs), and calcitonin are some of the medicines that have been approved. However, only a few studies have looked at how these medicines help people who take antiepileptic drugs (AEDs) lose their bone strength. They have been linked to bone damage. No one knows how AEDs and bone damage work together, even when anti-osteoporotic substances are used. Calcium vitamin D (CVD) supplements may benefit individuals who use AEDs for a long time, but they may not be suitable for everyone. Bisphosphonates can stop the bone loss caused by PHT in mice by making them less likely to get sick, which contains the bone loss caused by PHT (Khanna et al., 2011).

After a study called ADOPT was done, it found that schizophrenic men who took antiepileptic drugs had better bone mineral density (BMD) and no vertebral fractures after taking supplements for cardiovascular disease (CVD). They might not be able to use bisphosphonates since they have a multitude of troubles with their stomachs, as well as reports of a lot of bone turnover being slowed down and osteonecrosis of the jaw, which is when bone dies.

RLX, an approved medication for female osteoporosis and a SERM, is being tested in this study to see if it helps prevent or alleviate AED-induced bone deficits in female mice.

Raloxifene was our drug of choice for numerous reasons:

To begin, women may be more vulnerable to AED-induced bone turnover due to the increased risk of osteoporotic fractures throughout premature menopause and continuing trends.

Furthermore, the preponderance of AEDs, particularly PHT and SVP, may promote estrogen sleep disruption osteoporosis via the mechanisms discussed in the earlier paragraphs.

There have been a lot of studies done on levetiracetam in lab animals and RLX may make it easier for animals to have seizures.

To test if RLX had any effect on antiepileptic drug-induced bone alterations, it was compared to calcium and vitamin D3 (CVD) supplementation. Furthermore, raloxifene's impact on seizures and potential alteration of PHT and SVP's antiepileptic effectiveness were investigated. TGF-3 and estrogen may also have a role in the bone-damaging effects of AEDs and raloxifene.

1.24 PROBLEM STATEMENT OF THE THESIS

The thesis presented works on the problem on managing the pharmacological complications in AEDs induced degenerative disease which affects a large number of populations especially the females with more age number.

As well know that epilepsy is found to affect the nerves of brain and areas related to it and then it ultimately causes repeated, unprovoked epileptic seizures.

So, to treat these conditions of epilepsy and its neurodegeneration it is recommended to use Anti-Epileptic Drugs (AEDs) and use of these AEDs causes a voltage & frequency dependent decrease in conductance by attaching to the sodium channels in its inactivated state.

As per the norms on laboratory or practical level there is need of experimentation on the conditions related to it. For its process animal models of osteoporosis are conducted on female mice. But earlier the studies have been conducted on drugs like glucocorticoids or through the process of surgery where there was removal of ovaries from the mice takes place.

When we have studies the articles, we found that in mice very few studies have been done by looking at the effect of sodium valproate and LTM on bone mineral density and other indicators of bone health.

On the population basis when there is a very large intake of AEDs it has shown to reduce the bone density and bone mineral concentration women who were already suffering from post-menopausal osteoporosis and when they take AEDs for a longer period of time it has shown to lessen the bone mass and further break the bones by causing fractures.

Therefore, the drugs which stimulate cytochrome P450 enzyme system such as Phenytoin, Phenobarbital are linked to alter the bone metabolism and decrease the bone density and these kinds of fractures have come to be seen in women's who have reached menopause.

So, at last it concludes that the study will focus on the effect of AEDs on bone density and bone mineral concentration and how it alters the skeletal formation and then the drugs which can be used to treat these such pharmacological complications with lesser or no side effects in the body with further exploration of effectives of anti-seizures as per the stimulation of shock inducement as well as the effects of drugs on the mucoskeleton system of the body.

1.25 OBJECTIVES OF THE THESIS

The following objectives of this study are:

A study was conducted on female mice to compare the skeletal alterations caused by phenytoin, sodium valproate, and levetiracetam.

To see how raloxifene and calcium, and vitamin D3 supplementation affect bone density and bone metabolism caused by AED, test the effect in bone density and bone turnover caused by AED.

To explore the effects of tamoxifen on the antiseizure effectiveness of AEDs in the context of electroshock.

To see if testosterone and TGF-3 can help lessen the effects of AEDs or tamoxifen on the musculoskeletal system.

1.26 ORGANIZATION OF THE THESIS

The thesis presents are highly organized as per the norms and regulations provided for the research study. The thesis should initially focus on the title presented with its novelty and popularity according to the human needs.

The initials start with the Introduction of the topic given and all the information related to the topic should be complied in this part will full knowledge of all the terms used in the study.

Why?

That is under what reasons the thesis has been presented or what is the problem raised for which the study has been done and what are the reasons raised for the study as how much it is affecting the population's and what are the condition's raised due to which it is highly required for the research study.

Then to further process the study what are the raised objective required for the conduction.

Raised Objectives?

After there is clarification on what objectives we have to conduct the study there comes the process of Methodology required to conducting the study.

Enlist the materials required for the study in terms of chemicals, equipment's, reagents, instrumentation and all other necessary requirements for the study.

After all the process of methodoly then we have to collect all the data sone during the study process and when all the data is summarized then proceed for the results.

The results should be accurately complied for each of the study in the form of tables, charts, graph, figures and other methods.

Further all the results are disused under the topic Discussion.

At last, after all the finding has been taken and all the data have been verified then at last conclusion should be mentioned as a whole for the research study. It shows that when from the starting where we have started our work what was the condition for carrying out the research and what are the methods with their results are given.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The presented work is surveyed from various acknowledged sites such as PubMed, Google Scholar, Web of Sciences, Scopus, EMBASE, DOAJ and various others search engines were used for the articles study.

2.2 SOME RELATED LITERATURE REVIEW

As per Buket Tugan Yildiz et al., (2021) all the anti-epileptic drugs are known to be as long-term medications, whose side effects are seen commonly. A major but less known side-effects is the starting of metabolic bone disorders. The mechanism of this process is not known properly but is mainly seen as per use of cytochrome P450 enzyme -inducing anti-epileptics. But some recent studies showed the light on the pathogenesis of anti-epileptic metabolic bone diseases by the help of bone turnover markers.

By the work of C. Tamer Erel et al., (2010) the women with epilepsy undergoes and before process of menopause, in between 3 to5 years which depends upon frequency of seizures but for this less data is there. Data for the effects of the perimenopause & menopause on epilepsy are having conflicts as few studies showed that an increase in seizures with oral therapy along with conjugated equine estrogens & medroxyprogesterone acetate but for this no data is there for regimen. Women's starting the use of HT should be monitored properly as they are having anti-epileptic drugs which might change further. Herbal preparations are avoided as its efficacy is not certain and it might interact with the other AEDs.

The findings of Hosam k. Kamel (2006), as osteoporosis affected more than 20 million of people in the areas of north America which is responsible for 1.5 million fractures in U.S. Around 50% of white women in the U.S will have osteoporotic fractures in their whole life. Postmenopausal osteoporosis is the answer of estrogen deficiency that comes by up-regulation of many cytokines and more bone resorption. Many bones mineral density testing's methods are there, but WHO based diagnosis of post-menopausal osteoporosis on the presence of a BMD T-score which is 2.5 standard deviations or more below its mean for the young women as seen by X-ray absorptiometry at hip, spine, mid-radius. Further acknowledge proper calcium & vitamin D intake and it is aimed in preventing or treating postmenopausal osteoporosis. There are other non-pharmacological measures that modify the risk factors of this disease encompasses exercise, cessation of smoke, reducing consumption of caffeine and alcohol and avoid medicine that decrease bone mass.

The study of Alison m. Pack et al., (2004) it states that Anti-epileptic drugs are linked to many done diseases. Early reports showed that rickets in children & osteomalacia in adults that they are taken mainly in instutinalized people. Many studies on ambulatory adults and children's who are

taking AEDs do not show that rickets or osteomalacia but the reported abnormalities biochemical indexes of bone mineral metabolism and density. The fracture rates are increased in AED treated patients, the AEDs which induces cytochrome P450 enzyme system are commonly linked with the problems of brain. The data emerged showed that valproate which is an enzyme inhibitor also affects the bone. Many theories on the mechanism of AED- associated bone disease have been proposed but not a single one explained about is findings reported. Then by identifying the AED-treated patients which are at risk or having bone disease are important as many kinds of therapies are there.

Melania Martinez-Morillo., (2012) it states that there is not any agreement for the definition of osteoporosis in premenopausal women & diagnosis should be done properly that is not based upon densiometric parameters. It is also seen that there are other risk factors & history of fragility fractures, diseases or drugs which causes bone loss. For over 50% of pre-menopausal women having osteoporosis, they will also have a secondary cause that is remained diagnosed with its idiopathic osteoporosis. There are therapeutic considerations which are very less only by a few studies in these group of patients mainly for the risk of fractures. But on the other hand, the FRAX data which cannot be used to premenopausal women.

The work done by Naghme Adab et al., (2010) the women with epilepsy still about 0.6 % of pregnancies. Firstly, the potential for major structural malformations that follow gestational exposures for anti-epileptic's drugs is well known and causes concerns as how effectively epilepsy can be managed at the time of pregnancy. It is also focused that the structural and functional effects on the developing brain in completing others.

The study of Susanta Kumar Rout., (2010) as epilepsy is a condition in which the person feels recurrent seizures. The main area of treatment for epilepsy remains symptomatic rather than expansion in knowledge of its neurological disabilities. There are many pharmacological as well as surgical options which includes many different kinds of formulations. But there is a major disadvantage is the chronic side effects. Here the herbal drugs are targeted sides that show same mechanism as action like as the synthetic drugs. As allopathic drugs are introduced the use of crude drugs from the medicinal plants is on declining phase and this traditional knowledge will be lost in the near future. The novel anti-epileptic drugs are tolerated in better ways by the epileptic patients and on practical ground they are devoid on many known pharmacokinetic drug intractions.

The work of David W. Loring (2007)., the anti-epileptic drugs work as they reduces the irritability of neurons, that results in the un-desired side effects of reduced neuropsychological functions. With addition to it, many cognitive side effects of anti-epileptic drugs are associated with behavioral effects that might range from irritability and hyperactivity to some positive psychotropic effects on the mood. The patients with epilepsy undergo with neuro-psychological evaluations that are common for AEDs, as well is it is important for clinician recognition of the contributions of AEDs therapy to the neuropsychological profiles.
As per findings of Aaron M Cook et al., (2011) the disease epilepsy affects upto 1% of the general population and this further causes maximum disability. To manage seizures in patients with epilepsy it depends mainly upon the use of anti-epileptic drugs such as Phenobarbital, phenytoin, valproic acid are the primary medicines which are used in the treatment of epilepsy for many years.

The choice of AEDs depends upon the type of seizure, clinical activity spectrum, range of side effects & characteristics of patients. The AEDs having broad-spectrum activity are seen to shown an action at more than one molecular target.

The study of Ludmyla Kandratavicius et al., (2014) tells that the disease epilepsy is a chronic neurological disorder that is due to repeated seizures and affects millions of people across the globe. The comprehension of the difficult mechanisms that lies that epileptogenesis & seizures generation in the temporal lobe epilepsy & other forms cannot be completely acquired in the clinical studies with humans. That results in the use of proper animal models where some of the models replicated with the natural history of symptomatic focal epilepsy having an initial epileptogenic insult, that follows an apparent lateral period with that following a chronic spontaneous seizure. It is found that seizures are the combination of electrical as well as behavioral events that induces many alterations.

The work of Ramalakshmi Ramiah (2020), tells that epilepsy is a global issue that is affecting approx. 70 million people in the whole world population. Around 80% of which resides in the low- & middle-income countries with very less resources. However, many advanced treatments are there in some countries, where upto 90% of the people with epilepsy are not properly treated with the conventional anti-epileptic therapy in resources with limited countries.

The study of Sunila E.O' Connor et al., (2009) The women with epilepsy have some additional challenges as compared to the peers. The hormonal influences also increase the seizures activity while altering the endocrine functions and also affecting the fertility. In the population the use of anti-epileptic drugs reduces the efficacy of contraception methods as well as increases the risk of fetal malformations. There are some other pertinent issues in women with epilepsy as includes breastfeeding with bone mineral health.

The study of Vederhus J, Husebye ESN et al., (2023) this study undertakes the examination prevalence of self-reported experiences with the abuse in pregnant women's having epilepsy and the association between having experienced abuse with childbirth expectations. The method used in the study where a cross-sectional study of women having or not having epilepsy were enrolled with Norwegian mother, father & child cohort study 1999-2008. The data on the diagnosis of epilepsy, usage of anti-seizure medications, emotional, physical, sexual abuse with childbirth expectations were all summarized from the questionnaires that were completed at the time of gestational weeks 17-19 & 30 that further resulted that our study population include 295 women with ASM treated epilepsy, 318 women with ASM-untreated epilepsy and 93-94 women without epilepsy.

The work done by Hirak Kumar Mukhopadhyay et al., (2012) as now a days many people are facing many kinds of stress in the growing day to day life and most of the people in the world are suffering from many kinds of neurological conditions. The disease epilepsy is one of the most common neurological disorders of the brain that affects around 50 million individuals across the world & 90% of them are mostly from the developed countries. There are many types of seizures & having different mechanisms through which the brain generates seizures. The two hallmarks of the seizure's generation are the hyper-excitability of neurons & hyper-synchrony of neuronal circuits. A large variety of mechanisms changes the balance between excitation & inhibition to pre-dispose a complete spread of the brain to its hyper-excitability and its hyper-synchrony.

The work done by Emilio Perucca, (2021) says that the pharmacological armamentarium against epilepsy have wide spread over the last 3 decades, and presently involves around 30 different anti-seizure medications. Despite this there is around 1/3rd of people having epilepsy fails to achieve seizure freedom with present available medicines. This is further mitigated through the evidence that shows the clinical outcome for many people having epilepsy which has improved over many years. The physicians now have unprecedented opportunity in tailoring treatment choice according to the individual in accordance to maximize the efficacy & tolerability. So it tells about the advantages in the drug treatment of epilepsy in the last 5 years that focus on comparative effectiveness trials of second – generation drugs by introducing new pharmaceutical formulations in case of emergency.

The work done by Dieter Schmidth (2014), that the new drugs having shown more treatment options and some of which says that levetiracetam causes less drug interactions with less hyper-sensitivity as compared to the older ones. As such they do not reduce the prevalence of drug resistance epilepsy or prevents the development of epilepsy in patients who are at high risk. The development of anti-epileptic drugs presently needs to be revitalized so that we can discover a effective anti0seizure drug for the treatment of drug resistant epilepsy. The anti-epileptogenic agents help in preventing epilepsy before the first seizure in at risk patients & disease modifying agents in control ongoing severe epilepsy that is associated with progressive underlying diseases.

The study of Orcu Allahverdiyev et al., (2018) says that medicinal plants are more commonly used by folk in making infusions which are administered as herbal teas in pain relief & good health. There was investigation done in active components of plant extract by isolating, identifying its structure & its pharmacological effects and then finally utilizing it as a new agent from nature with few side effects & high economic value in the field of ethnopharmacology. With that to AEDs, that are currently used that suggested alternative therapies that are also able to minimize the seizures of epilepsy but the surgical intervention that still remains as the last option in the treatment of epilepsy.

The work done by Ebtesam Mohamed Fahmy et al., (2018) Serum calcium, phosphorus, Vitamin D were lower in comparison to it serum parathormone & alkaline phosphatase were higher in epileptic patients as compared to control subjects. The abnormalities in Bone mineral density were seen in 22 patients. A statics difference in DEXA scan measurements at different regions were found in between epileptic patients & control. The epileptic patients that are receiving enzyme inducer anti-epileptic drugs have less serum lever & lower BMD value as compared to those receiving enzyme inhibitors. The results of BMD were positively correlated with serum alkaline phosphatase & therapy duration.

The findings of Hueng-Chuen et al., (2016) epilepsy is a common neurological disorder across the world & anti-epileptic drugs are every time the first choice of treatment. Around more than 50% of patients with epilepsy who take AEDs shows bone abnormalities. Cytochrome P450 iso-enzymes are induced by AEDs mainly the classical AEDs, for example Benzodiazepines, carbamazepine, phenytoin, phenobarbital etc. The induction of CYP450 isoenzymes may cause Vit. D deficiency, hypocalcemia which increases risk of fracture by altering bone turnover that leads to impaired bine mineral density. The new AEDs such as levetiracetam, oxcarbazepine,

lamotrigine, topiramate shows broad spectra and are safe with better tolerance as then classical AEDs. The effects of AEDs on bone health are sometimes controversial.

The study of Marian Schini et al., (2023) bone turnover markers are mostly used in research as well as clinical practice. In the previous 20 years, more experience has been seen in measuring & interpreting these markers that includes commonly used bone formation markers & most commonly used resorption markers are serum C-telopeptides of type I collagen. Bone Turnover Markers are mainly measured by enzyme – linked immunosorbent assay. There are many sources that contributes to Bone turnover markers variability which includes uncontrolled factors & controlled factors especially by age, gender & controlled factors include fasting, feeding state, menstrual cycling & exercise.

There is study from Prabhu M.R et al., (2016) As we all know that bone is a dynamic organ that is made up of mostly collagen which provides structure to the skeleton & calcium phosphate which gives strength & hardness of the skeleton structure. Bone is constantly renewed by remodelling that is a lifelong process which constitute bone resorption & formation by repairing skeletal damage & maintaining calcium homeostasis. There are many hormones such as calcitonin, para-thyroid hormone, Vit. D, estrogen, testosterone and others for regulating resorption & formation. There are many factors such as age, low calcium diet, smoking & certain medicines as glucocorticoids, aromatase inhibitors, proton pump inhibitors& antiepileptic drugs can influence bone health that is used for longer duration treatment. Certain imaging techniques such as dual X-ray absorptiometry, Quantitative computerized tomography is seen for proper measurement of bone mineral density that helps to identify the risk of fractures. The newly bone biomarkers are found to increase its demand & show an important role in detecting bone loss at initial stages. There are recent evidences that suggest that these markers are used in combination with imaging techniques that helps in diagnosis of bone loss with use of main bone biomarkers that correlates with these drug classes causing bone loss at initial stage with treatment.

The study done by Samruddhi H. Charde et al., (2023) as seen in India there is a sizable share of female population in postmenopausal stage. The issues related to ageing in women are on increased risk of broken bones with a decrease in cortical & cancellous bone thickness as well as decrease in bone mineral density. The disease Osteoporosis has a very determined effect on the women's life, lower standard of living, decreased quality life & increased risk of fractures. There should be restriction on smoking & alcohol consumption. There are many pharmacological interventions which is done on patients that are diagnosed with this disease. The drug should be chosen on the side-effect's basis & its contraindications. It is difficult to follow up & patient compliance should be seen very carefully.

There is a study done by Remya Rajan et al., (2020) says that Osteoporosis is the most common metabolic bone disorder in human beings. It is very common in women and leads to chances to mortality & morbidity. Postmenopausal osteoporosis is due to removal of protective effects of estrogen at the time of menopause & increased follicle-stimulating hormone, this all leads to increased bone resorption. The evaluation of osteoporosis involves assessing risk factors, bio-chemical evaluation, assessing bone mineral density by the help of dual-energy X-ray absorptiometry, identification of vertebral fractures with vertebral fracture assessment tool & prediction of fracture risk with the help of different tools. The treatment includes prevention of osteoporosis by the help of different modifications in lifestyle by different tools. The treatment accommodates prevention of osteoporosis by the help of modification in lifestyle and

prevention of fall. There are many drugs licensed in reducing fracture risk mainly anti-responsive agents & anabolic agents.

The study of John A Sunyecz (2008), comments that Osteoporosis has many public health issues that causes mortality & morbidity. The uptake of Calcium & Vitamin D in the use of bone health is sometimes overlooked by patients and some healthcare providers. The approximate standard of care for osteoporosis should have proper intake of calcium & Vitamin D. There is some compliance to calcium & Vitamin D therapy that have a very good effectiveness in prevention of osteoporosis fractures. There s a recently algorithm presented (FRAX) that estimates an absolute fracture risk which allow the healthcare provider in deciding as when the pharmacological therapy is warranted addition to it Calcium & Vitamin D. There the pharmacologic therapy is advised there is regular use of calcium & vitamin D for reducing the risk of fractures. A bricks & mortar analog is helpful in counselling the patients

The work done by Hirak Kumar Mukhopadhyay et al., (2012) It is found that in the present days the people are facing many kinds of stress in the daily life and many of the people in the world are suffering from many kinds of neurological disorders. The disease Epilepsy is one of the most common neurological disorders of the brain that affects around 50 million of people across the world & in which 90% of them are from the developing countries. There are many patients with epilepsy that are suffering from severe emotional distress, behavioral disorders and isolation from the society. There are many types of seizures with different mechanisms through which the brain generates seizures. The two major hallmark of seizure generation is hyper-excitability of neurons & hypersynchrony of neural circuits.

The study of Pavlos P Lelovas et al., (2008) The disease Osteoporosis is a major systemic disorder that is affecting many Caucasian women. A large variety of animal species that includes rodents, rabbits, dogs etc. has been used as animal models in the research of osteoporosis. The present review study not only presents about the ovariectomized rats & its advantage is an effective model in the research of osteoporosis, with this it also provides information about the most relevant age & site of bone selection on the basis of its experimentation. With this, there are many methods of bone mass evaluation that are assessed as biochemical markers, densitometry, histomorphometry & bone mechanical testing that used for monitoring & evaluating these animal models in the prevention for osteoporosis.

The work of J. Barnsley et al., (2021) The disease Osteoporosis is a common chronic metabolic bone disorder that is related to mortality & morbidity. As the chances of osteoporosis increases with age that a paralleled elevation in the incidence fragility fractures also increases. The secondary cause of osteoporosis with that of osteosarcopenia is also discussed.

The work of Linda J Stephen (2019) shows that Epilepsy is a prevalence neurological disorder in the women across the globe. The hormonal changes that are occurring throughout the life of women can be influenced by a mechanism of seizures & anti-epileptic drugs that further shows many kinds of challenges. The effective contraception’s are mainly important for women with epilepsy of having child bearing potential as many anti-epileptic drug related teratogenicity & hormonal interactions. Many studies that shown that women do not receive contraception’s &

preconceptual counselling. The managing challenges in the population includes the higher changes of pregnancy complications.

The work of Tsung-Rong Kuo et al., (2017) suggest that Bone biomarkers includes formation, resorption & regulator released as the time of bone-remodelling process. These biomarkers have attracted major attention in the clinical assessment of osteoporosis treatment in the previous decades. By combining it with the measurement of bone mineral density the clinical applications of bone biomarkers have provided a comprehensive information for diagnosis of osteoporosis. With this, the analytical approach of bone biomarkers are in challenge for the future clinical studies.

The work done by Yoshiya Tanaka et al., (2005) says that bone homeostasis is maintained by the balance between bone resorption by osteoclasts & bone formation by osteoblasts. The osteoblasts not only play a major role in bone formation by synthesis of many bone matrix proteins but it also regulates maturation of osteoclasts by soluble factors & cognate interactions that results in bone resorption. This process mainly occurs at the interface in between proliferation synovium & bone tissue in rheumatoid arthritis. Therefore, the therapeutic strategies for these conditions, with an anti-TNF-alpha antibody & an IL-1 receptor antagonist that is effective intreating RA disease activity which also reduction in secondary osteoporosis & joint destruction. On the basis of improved understanding in immune signaling, investigation of suppression of cell functions might lead to improved understanding & proper treatment of diseases of bone metabolism & osteoporosis.

The work done by Valery Feigin et al., (2020) comments that Epilepsy is a major chronic disorder of the brain characterized by persisting pre-disposition in generating seizures, that is unproved by an immediate central nervous system insult, and also by neurobiologic, cognitive, psychological & social consequences of the recurrence in seizures. The disease epilepsy affects both the sexes & almost all ages across the world distribution. The prevalence & incidence of epilepsy is simple higher in mean in comparison to women and orders to enhance in the elderly people that reflects higher frequency of strokes, neuro-degenerative disorders & tumors in the group of people. The focal seizures are most common as compared to generalized seizures in children as well as adults. The etiology of epilepsy differs according to the socio-demographic characteristics of the affected people & the extent of the diagnostic work-up but as documented causes it is still lacking in around 50 % cases from higher income countries.

The study of Emmanouil Magiorkinis et al., (2014) states that by studying texts, medical books & reports along with a review of the data present on PubMed. The 19th century is marked by the work of French medical school & of John Hughlings Jackson that's et up research on epilepsy on the basis of solid scientific data. At the time of 20th century, the invention of EEG that was an advance in neurosurgery with discovery of anti-epileptic drugs & the delineation of the underlying pathophysiological mechanisms, they were the most significant advances in the area of research in epilepsy. In all the most confined connected physicians with epilepsy one can pinpoint on the work of Henry Gastaut, Wilder Penfield & Herbert Jasper. The recently advancement in the field of epilepsy includes development of advanced imaging techniques, development of microsurgery & the research on the connections in between genetic factors & epileptic seizures.

The study of Tracey A. Milligan (2021) states that the diagnosis & treatment of seizures & epilepsy is a normal task for every physician. Approx. 1 in every 10 people will have seizure in their lifetime. The disease Epilepsy have a tendency of unprovoked seizures. As Epilepsy is the 4th most common neurological disorder which affects 1 in 26 people in the region of united states & 65 million people worldwide. The evaluation of patients who are presenting seizures involves excluding an underlying neurologic or medical condition, classification of seizure type & determination of if the patient has epilepsy. There is need of proper treatment for accurate diagnosis of the type of epilepsy & syndrome & use of medication which is effective & does not have any kind of side effects. Most of the patients achieve complete control over seizure with the help of medication but if the medication is not successful the there is option of surgical treatment. There are special situations in the care of people with epilepsy that involves status epilepticus, women with epilepsy, older adult & safety issues.

The work done by Evangelis Giourou et al., (2015) states that the epilepsy disease affects 1% of the worlds population & it is the most common serious disorder of the brain that shows a great impact on the quality of life of the people affected with it mainly for those whose seizures are not controlled on complete basis. The disease Epilepsy have a multiplied origin & multifaced expression. It is mainly caused by a cluster of nerve cells in the brain that causes abnormality of signals which causes seizures. Anything that disturbs the normal pattern of neuronal activity which starts from illness to brain damage then to abnormal development of brain that further causes seizures. This disease develops due to abnormality in wiring of brain cells, imbalance of nerve signaling chemicals which are known as neurotransmitters these all changes in the brain cells are called as membrane receptors & channels.

The study of Emmanouil Magiorkinis et al., (2021) states that by thorough study of texts, medical books & reports with that review of all the literature present in PubMed was taken into under analysis. The 19th century is seemed by the works of the French medical school & of John Hughlings Jackson that set up the research on epilepsy on its major scientific basis. At the time of 20th century, the invention of EEG, advances in neurosurgery, discovery of anti-epileptic drugs & delineation of the present pathophysiological mechanisms there were the major advances in the areas of research in epilepsy. In all the major renounced physicians that were connected with epilepsy so one can pinpoint it through the work of Henry Gastaut, Wilder Penfield, Herbert Jasper. The major advances in the field of epilepsy includes development of advanced imaging techniques, development of microsurgery & research on the connection in between genetic factors & epilpetic seizures.

Tomasz A. Nowacki et al., (2016) This review will present the history and physical examination as the launching point of the first seizure evaluation, from the initial characterization of the event, to the exclusion of alternative diagnoses, and then to the determination of specific acute or remote causes. Clinical features that may distinguish seizures from alternative diagnoses are discussed in detail, followed by a discussion of acute and remote first seizure etiologies.

The study of Ettore Beghi., (2020) states that Epilepsy is a chronic disorder in the brain that is featured by pre-disposition in generating seizures which is unprovoked by an utmost insult of the central nervous system, and by neurobiologic, cognitive, psychological & social consequences of

the re-occurrence of seizures. The disease Epilepsy affects both males as well as females with all age groups around the world. The prevalence & incidences of epilepsy are little higher in men as compared to women and more in the elderly age group which reflects more chances of stroke, neurodegenerative disorders and tumors.

The work of DS Magazi et al., (2018) states that Epilepsy is a chronic disease which are building blocks of regularly taking seizures. At times it becomes a challenge in finding out its diagnosis. The treatment is not also same for this disease which makes it to depend on individual.

The work done by Lu Xia et al., (2017) states that while investigating the correlation in between initial response for anti-epileptic drugs & its long-term outcomes after 3 years in the patient who has newly diagnosed with epilepsy. The study method includes 204 patients with newly developed epilepsy that is further followed by 36 months. The long-term seizure freedom at 36 month was evaluated in the patient having seizure freedom 6 months or 12 months after starting treatment vs to that with no seizure freedom after the starting 6 months or 12 months. The unvitiated analysis & multiple logistic regression model are used in analyzing the association of potential confounding variables with the starting response to AEDs.

The study of N.E Bharucha., (2003) tells that there is high incidence, prevalence and treatment gap of disease epilepsy in India. As if defective methodology, the Indian studies have shown prevalence rates in comparison with from other countries. There is not any kind of incidence study exist. The prognosis for those having epilepsy is getting worse in the developing countries. The role of infection, mainly in neuro-cysticercosis has still worked out as the major contribution of its etiological factors. There is need of neuroimaging in calculating the risk of this disease. There are many reasons for the treatment gap that points out in India where people see various alternative forms of getting treatment on concurrent basis.

The study of Vinay kumari et al., (2014) conforms that Epilepsy is the common neurological disorder which does not see age, race, social status, gender etc. Around 5% of population in the world have got at least on seizure in the lives. At any point around 50 million people have epilepsy mainly in childhood, adolescence & older age. The main problem in epilepsy epidemiology in is accuracy with diagnosis. The diagnosis of epilepsy has more implication for physical, psychological and economical aspects. There should be proper measures required in reducing the incidence, ensuring proper treatment to all affected people & prevention of morbidity & mortality that is associated with epilepsy.

The work of Samuel Wiebe., (2014) tells that in fulfilling the task of informed clinical decision making & resource allocation, epidemiological studies in epilepsy should attach to the series of methodological standards. As seizure & epilepsy classification systems should be viewed in the extension of diagnosis as they have direct implications in the acquisition & interpretation of the epidemiologic data. The International League Against Epilepsy (ILAE) classification systems are also analysed. So, at last the lacking of Canadian epidemiological studies is also seen and the relevance & potential of this Canadian epidemiological data of epilepsy is also seen.

The study of Yash Srivastav et al., (2023) states that Epilepsy is a long-term medical disorder that occasionally causes unpredictable, unprovoked repeated seizures that shows impact on both

physical as well as mental abilities. This disease is among the most causing neurological problem. In Greek its terms as epilambanein, that is the root of the English word epilepsy that means “to be seized”. In this both sickness & the at time attack is meant through it. The word refers to the magical beliefs of time that leads to the stigma which is associated with epilepsy as people with epilepsy are seen to be dirty or bad. A present study found that around 90% of the 70 million epileptics worldwide live in the developing countries. The genetic testing has expanded the possibility of figuring out the aetiology of different types of epilepsies. So there is need of some prior clinical applications knowledge in completing this challenging endeavour.

The study of Joseph I. Sriven., (2015) conforms that Epilepsy is a disorder of unprovoked seizures which is a multifactorial disease that affects individuals of all the ages that particular predilection of the very young as well old. In addition to seizures, there are many patients that often reports about cognitive & psychiatric problems that are associated with both the seizures themselves as with its therapy. The disease epilepsy has many etiologies for both idiopathic & acquired with a huge range of therapeutic responses. In spite of many treatments that are available on controlling repetitive seizures which includes medications, diet, immunotherapy, surgery & neuromodulatory devices there is a large percentage of patients that continues in suffering the consequences of uncontrolled seizures that includes psycho-social stigma & death.

The work done by Valery Feigin et al., (2020) states that Epilepsy is a chronic disorder of the brain that is characterized by the continuing predisposition in generating seizures which is unprovoked by immediate insult of central nervous system and also by neurobiologic, cognitive, psychological & social consequences of reoccurrence of seizures. The disease epilepsy affects males as well as females across the globe. The prevalence & incidence of this disease is little bit higher in men as compared to women and peaks up in the elderly that shows more frequency of stroke, neurodegenerative diseases, tumors in all the age groups. The focal seizures are more common in comparison with generalized seizures in children as well as adults. The etiology of epilepsy differs according to the socio-demographic charactertics in the affected population & to the extent of the diagnosis area but a documented cause is still not there in around 50 % of the cases that are coming from high income countries.

The study of David Larsson MD et al., (2021) says that there is very few evidence for guiding the choice of anti-seizure medication in patients having poststroke epilepsy. There are some theoretical concerns about determination effects of anti-seizure medications in survival exist. There are enzyme-inducing drugs that interferes with the secondary stroke prevention. The US Food & Drug Administration comingly issued a safety announcement about the potential proarrhythmic property of lamotrigine. For investigating whether mortality varies with particular ASMs among patients with post-stroke epilepsy.

The study of Yun Sun et al., (2021) states that the human hereditary epilepsy is found to be related with mutation of ion channel in voltage-gated ion channels, ligand gated ion channels & G-protein coupled receptors. There are some transmembrane proteins or receptors gene which includes PRRT2 & nAChR & glucose – transporter genes are also related for the initials of epilepsy. The discovery of these all-genetic defects has led to more understanding of the pathogenesis of epilepsy.

The study done by V.V Potnis et al., (2020) states that at present time many people face different types of seizures in their daily life & most of the people in the world suffers from many kinds of neurological disorders. The disease Epilepsy is one of the most common neurological disorders of the brain that affects 50 million people around the world and 90% of them comes from developing countries. The genetic factors & brain infection, stroke, tumors & epilepsy causes high fever. It affects a major economic burden on the healthcare system in the area, workplace, school also homes. Most of the patients with epilepsy suffers from emotional stress, disorders of behaviour & continue isolation from society. There are many different kinds of seizures & mechanism by which the brain generates seizures.

The work done by Ana Paula de Arajo Boleti et al., (2024) states that the disease Epilepsy shows a condition where abnormal neuronal discharge occurs with synchronicity that shows a major public health challenge. There are many prognostic factors such as etiology, abnormality in electroencephalogram, type & number of seizures before treatment are the major factors for consideration. With this there are many 3rd generation anti-epileptic drugs that are presently available with many side-effects that negatively affects the quality of patient life. The inheritance & etiology of epilepsy is very difficult that involves many genetic & epigenetic mechanisms. There are different neurotransmitters that play a major role in establishing the normal physiology of other neurons. If there is dysregulation in neurotransmitters that is due to abnormal transmitter level in its receptor, that shows the seizures.

The work done by Yow Hui Yin et al., states the Epilepsy is among the common chronic neurological disorder that is affecting the individuals of all ages. With a good understanding of pathogenesis in epilepsy will finely provide the initial fundamentals in developing new anti-epileptic therapies that aims in preventing the epileptogenesis process with that treatment of epilepsy symptomatically. So, there are many investigations that embarks on advancing the knowledge of the mechanism present beneath epileptogenesis, understands the mechanism of pharmaco-resistance & discover anti-epileptogenic. There are animal models that play a major role in providing additional insights into the mechanism of epileptogenesis. Through these models this process has been seen to work for many molecular & biological pathways.

The study of D Smith et al., (2015) states that Epilepsy is a common and sometimes chronic condition having physical risks & consequences for psychological & socioeconomic factors that destroy the quality of life. The management of patients with epilepsy wants long term promise for general practitioner as well as the specialist. The main requirement is a full diagnosis, selection of treatment, proper counselling as per the needs of the individual. Many of the patients that comes for remission & might be discharged for the care of their general practitioner and remaining needs to continue their care in the developed clinic. At the time of course of patient condition they should be properly informed for taking the decisions about treatment choice, requirement of long-term treatment & options for tackling with the drug resistant condition along with its problems. Effectively this process involves cooperation in between the consultant & nurse specialist & the primary care physician. At present these facilities are not used now a days,

Emilio Perucca., (2021) The pharmacological armamentarium against epilepsy has expanded considerably over the last three decades, and currently includes over 30 different antiseizure medications. Despite this large armamentarium, about one third of people with epilepsy fail to achieve sustained seizure freedom with currently available medications. This sobering fact, however, is mitigated by evidence that clinical outcomes for many people with epilepsy have improved over the years. In particular, physicians now have unprecedented opportunities to tailor treatment choices to the characteristics of the individual, in order to maximize efficacy and tolerability. The present article discusses advances in the drug treatment of epilepsy in the last 5 years, focusing in particular on comparative effectiveness trials of second-generation drugs, the introduction of new pharmaceutical formulations for emergency use, and the results achieved with the newest medications.

The work done by GERALD LIU et al., (2017) states that the occurrence of a single seizure not always require pre-initiation of anti-epileptic drugs. There is risk of recurrent seizures should be guided for its use. In the adults, the factors are recurrence are the two unprovoked seizures that occurs for more than 24 hours apart, abnormal brain imaging, nocturnal seizures, epileptic syndrome that is associated with seizures. In children's, the key risk factors are abnormal results in electroencephalography, epileptic syndrome associated with seizures, severe head trauma, cerebral palsy. There are many risks of adverse effects from anti-epileptic drugs which is considerable & includes potential cognitive & behavioral effects. When no risk factor are present and due to reason that many of the patients do not require recurrence of seizures then physicians should consider that there should be a delay for giving anti-epileptic drugs until the time when second seizure again occurs. While delaying the therapy until the second seizures does not affect one to two years remission rates and at that time treatment should be started with monotherapy. The proper choice of medicine depends upon the type of seizure.

The work of Linda J Stephen MD et al., (2019) states that Epilepsy is a often occurring neurological condition in women across the globe. There are many hormonal changes that occurs in complete women life that is influenced by mechanism of seizure & anti-epileptic drugs that shows a unique challenge while its management. There is effective contraception that are important for women with epilepsy of childbearing potential as anti-epileptic drugs related teratogenicity & hormonal interactions as but many studies have reveled that women's do not receive contraceptive & preconceptual counselling. There are many managements challenge in the population which includes high risk of pregnancy complications & peripartum psychiatric problems that are seen in a woman without epilepsy. There is more research needed for clarifying the perfect role of folic acid supplementation in preventing congenital malformations in children who are born to women with epilepsy.

The work done by Harathi Panigrahi et al., (2014) states that an increasing number of infantile epilepsy syndromes are seen. But only particular number of infants (1-24 months) does not fit in any of the presently used subcategories. It presents the clinical presentation, electroencephalographic findings, evolution, management of some entities such as early infantile epileptic encephalopathy, early myoclonic epilepsy, infantile spasms, severe myoclonic epilepsy of infancy, generalized epilepsy with febrile seizures plus, hemi convulsions-hemiplegic-epilepsy, benign myoclonic epilepsy and many more.

The work done by Preksha P Sapariae., (2022) states that Epilepsy is a group of non-communicable neurological disease that is characterized by recurrent epileptic seizures. It is a condition that

causes unprovoked, recurrent seizures. It is known that seizure is a sudden rash of abnormal electrical activity in the brain. A disorder in which the activity of nerve cells in the brain gets disturbed which causes seizures. It also occurs as a result of genetic disorder such as trauma or stroke. At the time of seizure, a person gets abnormal behaviour, symptoms, sensations and this involves loss of consciousness.

The study of M D O Brien et al., (2005) states that there are many forms related to the management of epilepsy in women which is connected to its role in the reproduction process. In which some of them are also considered in adolescents whereas some are also related to pregnancy & some others are related with the menstrual cycle & the menopause. The presented review considers that contraception, fertility, teratogenicity & the use of folic acid which also consider the main investigation in pregnancy, hyperemesis, effect of pregnancy on the control of epilepsy, seizures effect on fetus, first fit in pregnancy, vitamin K, breastfeeding, counselling, catamenial epilepsy & bone-density.

Jeffrey D. Kennedy et al., (2019) Epilepsy is one of the most common neurologic conditions affecting women. Care for women with epilepsy requires expertise not only in seizure treatment, but also an understanding of the unique challenges presented in the treatment of women with epilepsy. Epilepsy and antiseizure medications (ASMs) interact with aspects of health care specific to women, including sexual and reproductive health, menstrual cycle, contraception and family planning, teratogenesis, and pregnancy. Knowledge of these issues allows providers to identify and anticipate circumstances in which epilepsy and its treatment overlap with topics of importance to women.

The study of Pallerla Srikanth et al., (2023) states that the disorder Epilepsy is a common neurological problem that causes neurobiological, cognitive, psychological consequences. These impairments lead to substantial social influences in the women in their whole life cycle stages mainly which is connected with the stigma, severity of illness, quality of life with many other psychological problems which are added to reproductive decision-making associated factors in the women with this disease. Then a cross- sectional descriptive study was done for assessing the knowledge, risk perception, reproductive decision-making factors with the women with this disease. Then a semi-structured questionnaire was created for collecting the socio-demographic & clinical charactertics of around 49 women who took the treatment of epilepsy from a national tertiary referral care centre for neuro-psychiatry in the region of South India. Then the researcher prepared a 24-item questionnaire that includes 3 open – ended questions for assessing the knowledge about the enhanced heath issues & epilepsy.

The work done by B.A Leeman et al., (2007) states that the use of diagnostic tools & treatment options for the disease epilepsy has expanded in the present years. The use of Imaging techniques at a time conformed to the research laboratories that these are a routinely used processes for the clinical purposes. The medications that are unavailable for a few years ago is the present time used as a first-line agent. The patients with refractory seizures lead forward for earlier surgical interventions that consider the treatment with the use of medical devices & properly seen for non-pharmacological alternatives.

Lawrence G. Seiden et al., (2021) At when a new anti-seizure medication is taken, a ‘‘start low, go slow” titration approach is generally recommended and has been shown to reduce the risk of

severe idiosyncratic reactions with certain medications and improve tolerability with regard to many frequently occurring central nervous system-related adverse effects (e.g., somnolence, dizziness). Many patients with epilepsy will require medication changes due to lack of efficacy or intolerability of the initial regimen. When this occurs, patients may be switched from one monotherapy to another or receive adjunctive therapy. When transitioning a patient from one ASM to another (referred to as monotherapy conversion or transitional polytherapy), there are several strategies for tapering the baseline ASM depending on the clinical scenario. Regardless of the particular strategy, the goal should be to discontinue the baseline ASM in order to prevent increased toxicity due to drug load. When adding on ASM therapy, flexible titration of the new ASM and adjustment of concomitant ASMs to achieve disease control with the lowest possible drug load (lowest numbers and lowest doses) may help improve tolerability of the add-on therapy. Communication with patients during the initiation of a new therapy may help patients adhere to the titration schedule, allowing them to reach their optimal maintenance dose.

Aaron M Cook et al., (2011) Epilepsy affects up to 1% of the general population and causes substantial disability. The management of seizures in patients with epilepsy relies heavily on antiepileptic drugs (AEDs). Phenobarbital, phenytoin, carbamazepine and valproic acid have been the primary medications used to treat epilepsy for several decades. Since 1993 several AEDs have been approved by the US FDA for use in epilepsy. The choice of the AED is based primarily on the seizure type, spectrum of clinical activity, side effect profile and patient characteristics such as age, comorbidities and concurrent medical treatments. Those AEDs with broad-spectrum activity are often found to exert an action at more than one molecular target. This article will review the proposed mechanisms of action of marketed AEDs in the US and discuss the future of AEDs in development.

S. Svalheim et al., (2011) Patients with epilepsy have a 2–6 times greater risk of bone fractures compared with the general population. There are several potential explanations. Some fractures are caused by seizure-related injuries, or they may be associated with the osteopenic effect of reduced physical activity in patients with epilepsy. Antiepileptic drugs (AEDs), especially those that affect the liver enzymes, e.g., phenytoin, carbamazepine, phenobarbital, as well as valproate, are also associated with increased fracture rate and low bone mineral density. Many patients with epilepsy and general practitioners seem unaware of this problem. Measurements of bone density should be taken regularly in patients at risk of developing osteoporosis. Non-pharmaceutical initiatives, such as partaking in regular physical activity and eating a well-balanced diet, should be recommended. The risk of developing osteoporosis should be taken into consideration in the selection of an AED for treating a newly diagnosed patient with epilepsy.

Christian Meier et al., (2011) In recent years there has been increasing evidence suggesting that epilepsy and its treatment can have adverse effects on bone mineralization and calcium metabolism. Many studies have shown a significant reduction in bone mineral density (BMD) and an increased fracture risk in patients treated with enzyme-inducing antiepileptics (phenobarbital, carbamazepine, phenytoin). It is assumed that CYP450-inducing antiepileptic drugs (AEDs) upregulate the enzymes which are responsible for vitamin D metabolism, with the effect of converting 25(OH) vitamin D into inactive metabolites, resulting in reduced calcium absorption with consecutive secondary hyperparathyroidism. Data on bone-specific effects of newer AEDs are limited; nevertheless, alterations of bone metabolism have been reported for oxcarbazepine, gabapentin and, in preclinical studies, for levetiracetam. Prophylactic administration of adequate

amounts of calcium and vitamin D is recommended for all patients. For patients with long-term AED exposure, BMD measurement is recommended as part of osteoporosis investigation (especially for patients treated with enzyme-inducing AEDs and where there are major risk factors for fractures). Drug therapy (bisphosphonates) is reserved for the treatment of patients who have a high fracture risk; there are no specific intervention studies available in patients with epilepsy.

Alison M. Pack et al., (2004) Antiepileptic drugs (AEDs) are associated with bone disease. Early reports found rickets in children and osteomalacia in adults, but those reports were primarily in institutionalized persons. Studies in ambulatory adults and children taking AEDs do not reveal rickets or osteomalacia but do report abnormalities in biochemical indexes of bone mineral metabolism and density. In addition, fracture rates are increased in AED-treated patients. AEDs that induce the cytochrome P450 enzyme system are most commonly associated with abnormalities in bone. Emerging data suggest that valproate, an enzyme inhibitor, may also affect bone, and there is limited information on the newer AEDs. Several theories on the mechanism of AED-associated bone disease have been proposed, but no single one explains all the reported findings. Identifying AED-treated patients who are at risk for or have bone disease is important, as multiple therapies are available.

Enamul Kabir et al., (2023) Epilepsy is a common medical and social disorder characterized by epileptic seizures. These seizures occur due to disruptions of electrical signals in the brain. Epilepsy also characterized by the neurobiologic, cognitive, psychological, and social consequences of this condition. Aim of the study: The aim of the study was to find out the Association between Antiepileptic Drugs and Therapy with Bone Mineral Density (BMD). Methods: This study was conducted in the Saleh Child Development and Disability Management Center (SCDDMC), of Institute of Child and Mother Health (ICMH) Matuail, Dhaka. A total of 31 children's age ranged 5-15 years diagnosed as epilepsy on the basis of both clinical examination and investigation (EEG) receiving antiepileptic drugs (AEDS) for at least two years were recruited in this study. Result: The mean serum calcium was 9.22 ± 0.78 mg/dl, and the mean BMD score of the spine was 0.66 ± 0.14. The mean BMD of the left femur neck was 0.66 ± 0.15. The name and combination of drugs used in the study children. It was found that 35.5% of the children received PB alone, followed by 16.1% receiving VPA alone. 12.9% received PB with VPA, 12.9% received PB with VPA with others, 9.7% received VPA with NTZ, and 6.5% received PB with VPA with NTZ and VPA alone.

Mufeed Akram Taha et al., (2024) Epilepsy is a common chronic neurological disorder that affects almost 50 million people worldwide. Most patients require long-term, and sometimes lifelong therapy with antiseizure medications (ASMs), our case control study evaluates the long-term effect of carbamazepine (CBZ) and valproate (VPA) on bone mineral density and bone health biochemical markers. 50 patients with newly diagnosed epilepsy who had no history of antiseizure medications (ASMs) intake prior were divided into 2 groups. In the first group, 25 patients started carbamazepine (CBZ) monotherapy, while the second group included 25 patients who started receiving valproate (VPA) monotherapy. Another 25 healthy individuals were considered as a control group. The primary outcome of our study is to evaluate the percentage of osteoporosis compared to the duration of ASM therapy measured by the T score at two different sites in the body at baseline, after 3 and 6 months of therapy, while measurements of bone biomarker variables are considered secondary outcomes.

Parisa Delkash et al., (2020) Osteoporosis is the most common skeletal disease in humans in which bone density and quality are reduced, leading to osteoporotic fractures. Most osteoporotic fractures occur in areas such as the spine, hip, and wrist. Factors involved in the development and exacerbation of osteoporosis include non- modifiable factors such as age, gender, race, and genetics, and modifiable factors such as body mass, smoking, sedentary lifestyle, and diet. In this review article, we have evaluated osteoporosis, the related factors, and the management of this disease. During this study, after electronic search and review of titles and abstracts, articles with appropriate consistency and content were included in the study. The findings showed that although a decrease in bone density and strength with age is inevitable, by taking measures such as the use of pharmacological and non-pharmacological strategies, increasing physical activity, and adding nutrition containing vitamin D in the diet of people, the occurrence of this complication can be prevented.

J Barnsley et al., (2021) Osteoporosis, a common chronic metabolic bone disease is associated with considerable morbidity and mortality. As the prevalence of osteoporosis increases with age, a paralleled elevation in the rate of incident fragility fractures will be observed. This narrative review explores the origins of bone and considers physiological mechanisms involved in bone homeostasis relevant to management and treatment. Secondary causes of osteoporosis, as well as osteosarcopenia are discussed followed by an overview of the commonly used pharmacological treatments for osteoporosis in older people.

Nader Salari et al., Osteoporosis affects all sections of society, including families with people affected by osteoporosis, government agencies and medical institutes in various fields. For example, it involves the patient and his/her family members, and government agencies in terms of the cost of treatment and medical care. Providing a comprehensive picture of the prevalence of osteoporosis globally is important for health policymakers to make appropriate decisions. Therefore, this study was conducted to investigate the prevalence of osteoporosis worldwide. A systematic review and meta-analysis were conducted in accordance with the PRISMA criteria. The PubMed, Science Direct, Web of Science, Scopus, Magiran, and Google Scholar databases were searched with no lower time limit up till 26 August 2020. The heterogeneity of the studies was measured using the I^2 test, and the publication bias was assessed by the Begg and Mazumdar's test at the significance level of 0.1.

Eliyahu Greenberg., (2022) Osteoporosis is a disease of the skeleton that becomes more common with advanced age, especially in postmenopausal women. Osteoporosis increases the risk of fractures, thereby reducing the quality of life for those who suffer from it. Due to the aging population, direct costs resulting from osteoporosis are projected to reach upward of $25 billion per year by 2025. The main pharmaceuticals primarily target osteoclasts. Exercise may be an effective method of preventing osteoporosis, although more research needs to be done. More research should be conducted to explore potential ways to enhance osteoblastic activity as a method to treat and/or reverse osteoporosis. This review compares the pros and cons of major methods to treat osteoporosis.

Suman VB et al., (2013) Osteoporosis is a global problem affecting 150 million men and women worldwide. Osteoporosis is a condition characterized by decreased bone strength. Women are four times likely to develop osteoporosis than men. It is prevalent in post-menopausal women but also occurs in men and women with underlying conditions or major risk factors associated with bone demineralization. Its chief clinical manifestations are vertebral and hip fractures, although fractures can occur at any skeletal site. Osteoporosis ranks as one of the 5 costliest diseases of aging after diabetes, hyperlipidemia, hypertension and heart diseases. As age advances, the incidence of osteopenia and osteoporosis increase and with the progressive aging of the world's population, there will be a resultant increase in the osteoporotic fractures. It is a matter of great concern that although the effects of osteoporosis are seen in the elderly population particularly women, the roots of osteoporosis are laid much earlier in life. Thus, osteoporosis has been described as a condition dealt with by geriatrician but with roots in pediatrics. We are trying to elaborate on risk factors, measurement of BMD and management of osteoporosis.

David B.N Lim et al., (2023) Childhood osteoporosis leads to increased propensity to fracture, and thus is an important cause of morbidity, pain and healthcare utilization. Osteoporosis in children may be caused by a primary bone defect or secondary to an underlying medical condition and/or its treatment. Primary osteoporosis is rare, but there is an increasing number of children with risk factors for secondary osteoporosis. Therefore, it is imperative that all paediatricians are aware of the diagnostic criteria and baseline investigations for childhood osteoporosis to enable timely referral to a specialist in pediatric bone health. This review will discuss the approach to diagnosis, investigation and management of childhood osteoporosis, with particular consideration to advances in molecular diagnosis of primary bone disorders, and current and emerging therapies for fracture reduction.

Manoj Chadha et al., (2022) Osteoporosis is a global health disease. Increasing life span will add to the burden of osteoporosis, especially in postmenopausal women. The lifetime risk of osteoporotic fractures is 30% to 40%. Fractures pose an extensive burden on healthcare resources. Therefore, early diagnosis of osteoporosis is necessary. Methods: In this review, we provide a comprehensive approach to the current epidemiology, diagnosis aspects, treatments and fracture management in relation to the osteoporosis. Results: In assessing osteoporotic patients, good medical history with identification of clinical risk factors should be done. Along with basic blood investigations, bone mineral density, vertebral imaging, and bone turnover markers can aid the accurate diagnosis of bone loss. Modification of risk factors and dietary interventions are the first step in managing osteoporosis. Multiple options can be tailored to the individual needs in the treatment of osteoporosis. The frequency and duration for which the treatment is continued depend on the individual response to treatment.

Rubanpal Khinda et al., (2022) The prevalence and predictors of osteoporosis and osteopenia remain to be examined in the postmenopausal women of Punjab, India. The present cross-sectional study screened 1628 postmenopausal women during September 2019 to March 2020. Osteoporosis and osteopenia were confirmed on the basis of T-scores using dual energy X-ray absorptiometry (DXA) at the hip (femoral neck) and lumbar spine regions (L1–L4 vertebrae). The prevalence of osteoporosis and osteopenia was observed to be 30.50% and 44.20%, respectively, in postmenopausal women of Punjab. In univariable and multivariable regression analysis, variables independently influencing the risk of osteoporosis and osteopenia were: higher systolic blood

pressure (95%CI: 1.22–3.11 & 1.08–2.49), triglyceride levels (95%CI: 1.21–3.10 & 1.42–2.51), poor sleep quality (95%CI: 1.91–2.47 & 1.76–3.47) and C-reactive protein levels (95%CI: 2.18–3.56 & 1.03–2.18). Years since menopause >10 years was observed to be an independent predictor for the risk of osteopenia but not for osteoporosis. Higher body mass index (>30 $kg \cdot m^{-2}$) was observed to be a significant protective factor against the risk of osteoporosis (95%CI: 0.26–0.68) and osteopenia (95%CI: 0.19–0.52). The higher prevalence rates of osteoporosis and osteopenia in postmenopausal women of Punjab are alarming, which solicits awareness and earlier testing of those women who are approaching menopause.

Sushrut Babhulkar et al., (2021) Aim of the study was to determine the prevalence of bone loss (both osteopenia and osteoporosis) at national and regional levels in India. Methods: In this retrospective study, data obtained from in-clinic screening camps conducted for bone loss was analysed. Participants were apparently healthy adults (aged 18 years and above) evaluated for bone mineral density (BMD) using calcaneal quantitative ultrasound (QUS) of left foot. Based on t score of BMD obtained, participants were labelled as normal (T-score <-1 SD), osteopenia (t score-1 to -2.5 SD) and osteoporosis (t score <-2.5 SD). Results: In total, data of 31238 participants was analysed retrospectively. Mean age was 47.8±14.2 years and 47.6% were females. Among females, 38.8% were postmenopausal women (age >50 years). Overall prevalence of osteopenia and osteoporosis was 49.9 and 18.3% respectively. Across East, West, North and South India, the prevalence of osteopenia was 51.3, 47.9, 55.6 and 47.4% respectively whereas prevalence of osteoporosis was 18.4, 16.3, 16.4 and 20.7% respectively. Prevalence of osteoporosis was slightly higher in females than males (19.4 vs 17.3%). Among postmenopausal women, overall osteoporosis prevalence was 33.1% and ranged from 16.9% in North region to 21.8% South region. Prevalence of osteoporosis (37.0 vs 12.5%) was higher in elderly (≥60 years) than adults.

David B Burr et al., (2004) Bone is organized in a hierarchical way that enables it to perform its mechanical, metabolic/endocrine, hematopoietic, and protective functions. At the nanoscale level, bone is a composite material composed of collagen, mineral, water, and a host of noncollagenous proteins. At the microstructural level, bone can be lamellar or nonlamellar, and can be formed either de novo, by direct apposition (primary), or by replacing existing bone (secondary). Macroscopically, bone is compact (cortical), with low porosity, or cancellous, with high porosity. This creates several surfaces from which bone can either be removed or added; these are termed the four skeletal envelopes (endocortical, periosteal, trabecular, intracortical). Because it is a dynamic living entity, bone also has its own special vascular supply and innervation. These features, together with the amount of bone and the quality and organization of the tissue, provide bone with its strength and fracture resistance.

Agnes Berendsen et al., (2015) The development of the vertebrate skeleton reflects its evolutionary history. Cartilage formation came before biomineralization and a head skeleton evolved before the formation of axial and appendicular skeletal structures. This review describes the processes that result in endochondral and intramembranous ossification, the important roles of growth and transcription factors, and the consequences of mutations in some of the genes involved. Following a summary of the origin of cartilage, muscle, and tendon cell lineages in the axial skeleton, we discuss the role of muscle forces in the formation of skeletal architecture and assembly of musculoskeletal functional units. Finally, ontogenetic patterning of bones in response to

mechanical loading is reviewed. This article is part of a Special Issue entitled "Muscle Bone Interactions".

Alizae Marny Mohamed., (2008) Bone is a specialised connective tissue and together with cartilage forms the strong and rigid endoskeleton. These tissues serve three main functions: scaffold for muscle attachment for locomotion, protection for vital organs and soft tissues and reservoir of ions for the entire organism especially calcium and phosphate. One of the most unique and important properties of bone is its ability to constantly undergo remodelling even after growth and modelling of the skeleton have been completed. Remodelling processes enable the bone to respond and adapt to changing functional situations. Bone is composed of various types of cells and collagenous extracellular organic matrix, which is predominantly type I collagen (85–95%) called osteoid that becomes mineralised by the deposition of calcium hydroxyapatite. The non-collagenous constituents are composed of proteins and proteoglycans, which are specific to bone and the dental hard connective tissues. Maintenance of appropriate bone mass depends upon the precise balance of bone formation and bone resorption which is facilitated by the ability of osteoblastic cells to regulate the rate of both differentiation and activity of osteoclasts as well as to form new bone. An overview of genetics and molecular mechanisms that involved in the differentiation of osteoblast and osteoclast is discussed.

Tsung -Rong Kuo et al., (2017) Bone biomarkers included formation, resorption and regulator are released during the bone remodeling processes. These bone biomarkers have attracted much attention in the clinical assessment of osteoporosis treatment in the past decade. Combination with the measurement of bone mineral density, the clinical applications of bone biomarkers have provided comprehensive information for diagnosis of osteoporosis. However, the analytical approaches of the bone biomarkers are still the challenge for further clinical trials. In this mini-review, we have introduced the functions of bone biomarkers and then recently developed techniques for bone biomarker measurements have been systematically integrated to discuss the possibility for osteoporosis assessment in the early stage.

Jamea A. Yaeger., (1963) It introduces this monograph, there is a quite thorough discussion of the gross and microscopic descriptions of bone growth and remodeling published during the 19th century. However, the bibliography becomes increasingly selective as recent decades are approached. The more recently described and clinically important relationships between bone remodeling and the chemical equilibrium between blood and bone are not discussed. The body of the monograph describes several types of mammalian cortical bone which are discernible with low power light microscopy. The development of these bone types is discussed, using long bones as examples. Although Enlow's descriptions are more profusely illustrated, I found these "principles of remodeling" as well explained in Lacroix's *The Organization of Bones*.

Harjeet Kaur et al., (2016) Epilepsy is the most common neurological disorder which significantly affects the quality of life and poses a health as well as economic burden on society. Epilepsy affects approximately 70 million people in the world. The present article reviews the scientific rationale, brief pathophysiology of epilepsy and newer antiepileptic drugs which are presently under clinical development. We have searched the investigational drugs using the key words ‘antiepileptic drugs,’ ‘epilepsy,’ ‘Phase I,’ ‘Phase II’ and ‘Phase III’ in American clinical trial, the relevant published articles using National Library of Medicine's PubMed database, company websites and

supplemented results with a manual search of cross-references and conference abstracts. his review provides a brief description about the antiepileptic drugs which are targeting different mechanisms and the clinical development status of these drugs. Besides the presence of old as well as new AEDs, still there is a need of new drugs or the modified version of old drugs in order to make affected people free of seizures. An optimistic approach should be used to translate the success of preclinical testing to clinical practice. There is an urgent need to improve animal models and to explore new targets with better understanding in order to develop the novel drugs with more efficacy and safety.

S. Aneja et al., (1996) During the past few years, a number of drugs have been added to the anti-epileptic arsenal. This review focusses on five of these drugs which have undergone extensive trials: Vigabatrin, Lamotrigine, Gabapentin, Felbamate and Oxcarbazepine. Some of these antiepileptic drugs appear to be helpful for treatment of catastrophic childhood epilepsies. Vigabatrin appears promising in children with infantile spasms who do not respond to ACTH or Prednisolone. Children with Lennox-Gastaut syndrome may respond to treatment with lamotrigine or Vigabatrin. Gabapentin and vigabatrin have proved to be effective in refractory partial seizures. Oxcarbazepine, a keto derivative of carbamazepine, is as effective as Carbamazepine but has a better safety profile. Lesser neurotoxicity and fewer drug interactions is another advantage with these drugs. However, monitoring is required to determine the long-term safety with their usage. These drugs have a definite role in childhood epilepsies refractory to conventional antiepileptic drugs.

Josemir W. Sander., (2004) Up to 70% of people developing epilepsy may expect to become seizure free with optimum antiepileptic drug (AED) therapy. The remaining 30% are the most difficult to treat. Most patients are controlled on a single AED, but a small proportion requires a combination of two agents. Add-on therapy with a second drug, rather than substitution, may be a viable and rational approach in some patients, particularly if the first drug is relatively well tolerated. Precise classification of the type of seizures, as well as the epilepsy syndrome, together with careful recording of both seizures and adverse effects, are essential if rational management decisions are to be made. The goal of therapy should be complete seizure freedom with a single drug taken once or twice a day and without adverse effects. If control is difficult to achieve, the maximum tolerated dose of each drug should be explored, but a balance needs to be struck between adverse effects and control of seizures.

Susanta Kumar Rout et al., (2010) Epilepsy is a condition in which a person has recurrent seizures. The mainstay of treatment for epilepsy remains symptomatic despite the rapid expansion in knowledge of its neurological disabilities. Therapeutic options, both medical, surgical and non-medical have been markedly improved over the past decades, resulting in better condition, activities of daily living, and quality of life for epileptic patients. The principle of seizure (Epilepsy) management should be individualized and the selection of treatments should aim to control symptoms as well as to prevent other complications. Various pharmacologic and surgical options are available, including different formulations. There are number of drugs available for treatment of epilepsy in modern therapy. But the major disadvantages being faced are their chronic side effects. Herbal drugs are acting at target side having same mechanism of action as that of synthetic drugs. With the introduction of allopathic drugs, the use of crude drugs from medicinal plants is on the decline and subsequently this traditional knowledge may be lost in the near future.

Novel antiepileptic drugs are better tolerated by epileptic patients and practically are devoid of important pharmacokinetic drug interactions.

Nadia Rucci., (2008) Bone remodelling is an active and dynamic process that relies on the correct balance between bone resorption by osteoclasts and bone deposition by osteoblasts. Moreover, these two functions must be tightly coupled not only quantitatively, but also in time and space. When the coupling is lost, the correct bone mass could be compromised, leading to several skeletal pathologies. Indeed, bone loss and osteoporosis are the result of an increased osteoclast function and/or a reduced osteoblast activity. In contrast, other pathologies are related to osteoclast failure to resorbe bone, such as osteopetrosis, a rare genetic disorder characterized by an increased bone mass and also linked to an impairment of bone marrow functions. Starting from these assumptions, it is necessary to more deeply understand the molecular mechanisms regulating bone cell functions. Indeed, recent studies evidenced a complex interplay between the immune and skeletal systems, which share several regulatory molecules including cytokines, receptors and transcription factors. These data allowed to more deeply understand the mechanisms underlying bone mass regulation and could open new avenue to identify target molecules for alterantive therapies more efficacious against bone diseases.

Erik Fink Eriksen., (2010) Bone remodeling is a tightly regulated process securing repair of microdamage (targeted remodeling) and replacement of old bone with new bone through sequential osteoclastic resorption and osteoblastic bone formation. The rate of remodeling is regulated by a wide variety of calcitropic hormones (PTH, thyroid hormone, sex steroids etc.). In recent years we have come to appreciate that bone remodeling proceeds in a specialized vascular structure, —the Bone Remodeling Compartment (BRC). The outer lining of this compartment is made up of flattened cells, displaying all the characteristics of lining cells in bone including expression of OPG and RANKL. Reduced bone turnover leads to a decrease in the number of BRCs, while increased turnover causes an increase in the number of BRCs. The secretion of regulatory factors inside a confined space separated from the bone marrow would facilitate local regulation of the remodeling process without interference from growth factors secreted by blood cells in the marrow space.

Thinn Hlaing et al., (2014) Bone turnover markers of resorption and formation are released during the process of bone remodelling. These markers have been extensively studied in a number of therapeutic trials of osteoporosis during the past decade. This has led to better understanding of their physiology, clinical applications and possible ways to optimize analytical techniques. Bone markers can complement the results of bone mineral density in the management of osteoporosis, but their use in clinical practice is challenged by pre-analytical and analytical variability. This review will discuss different types of bone markers, their limitations, use in different metabolic bone diseases and current recommendations from the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine bone marker standards working group.

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Pierre D. Delmas., (1993) The noninvasive assessment of bone turnover has received increasing attention over the past few years because of the need for sensitive markers in the clinical investigation of osteoporosis. Markers of bone formation include the serum measurement of total and bone-specific alkaline phosphatase, osteocalcin, and type I collagen extension peptides. Assessment of bone resorption can be achieved with measurement of fasting urinary calcium and hydroxyproline, urinary hydroxylysine glycosides, urinary excretion of the pyridinium cross-links (pyridinoline and deoxypyridinoline), and plasma tartrate-resistant acid phosphatase activity. Several studies performed in a variety of metabolic bone diseases have shown these markers to be of unequal sensitivity and specificity. In addition, some of them are not fully characterized. For assessment of the level of bone turnover in women with vertebral osteoporosis, serum osteocalcin and urinary pyridinoline appear to be the most sensitive markers so far. Programs combining bone mass measurement and assessment of bone turnover by several markers in women at the time of menopause are being developed in an attempt to improve the assessment of the risk for osteoporosis. Efforts are being made to develop more convenient assays and to identify other markers of bone turnover. A battery of various specific markers is likely to improve the assessment of the complex and subtle abnormalities of bone metabolism that characterize metabolic bone diseases, especially the various aspects of osteoporosis.

Fredeick R. Singer et al., (2024) Biochemical markers of bone turnover provide clinically useful evidence of the normal and pathologic processes that reflect bone cell activity in the skeleton. Understanding the behavior of markers of bone formation and bone resorption should aid in managing patients with a variety of skeletal disorders.

Kiyoshi Sekiya., (2023) Osteoporosis, a prevalent skeletal disorder, poses significant challenges to bone health globally, particularly affecting aging populations and postmenopausal women. This article provides a comprehensive overview of osteoporosis, exploring its causes, symptoms, risk factors, prevention strategies, and available treatments. The intricate balance of bone remodeling, influenced by hormonal changes, aging, genetics, nutritional deficiencies, and lifestyle factors, plays a pivotal role in the development of osteoporosis. While the condition often progresses silently, leading to fragility fractures, understanding its symptoms, such as back pain, height loss, and fractures, is crucial for early detection. Numerous risk factors, including age, gender, family history, race, and body size, contribute to an individual's susceptibility. Prevention strategies encompass proper nutrition, physical activity, avoidance of smoking and excessive alcohol, and regular health checkups. In the event of a diagnosis, treatments range from medications, lifestyle modifications, and physical therapy to surgical interventions, aiming to slow bone loss, enhance bone density, and mitigate fractures. This abstract emphasizes the multifaceted nature of osteoporosis and underscores the importance of proactive measures, including regular screenings and a holistic approach to bone health, to mitigate its impact and promote overall well-being.

Jose R. Caerio Rey et al., (2009) Raloxifene, a member of the class of selective estrogen receptor modulators (SERM), reproduces the beneficial effects of estrogens on the skeletal systems, without the negative effects estrogens on breast and endometrium. This is a review article summarizing its mechanism, effects on bone and its applicability in traumatology clinical practice. In postmenopausal osteoporosis, this drug has been proven to decrease accelerated bone turnover, increase bone mineral density (BMD), and to structurally recover bone, decreasing the risk of vertebral fractures and the risk of non-vertebral fractures in patients with previous, severe vertebral fractures. Moreover, raloxifene appears to lower the risk of invasive breast cancer. Raloxifene would be efficacious in the prevention and treatment of postmenopausal osteoporosis.

Enrico M. Messalli et al., (2010) The integrity of bone tissue and its remodeling that occurs throughout life requires a coordinated activity of osteoblasts and osteoclasts. The decreased estrogen circulating level during postmenopausal transition, with a prevalence of osteoclastic activity over osteoblastic activity, represents the main cause of bone loss and osteoporosis. Osteoporosis is a chronic disease requiring long-term therapy and it is important to evaluate the efficacy and safety of treatments over several years, as the fear of health risks is a common reason for discontinuing therapy. Raloxifene is a selective estrogen receptor modulator (SERM) leading to estrogen-agonist effects in some tissues and estrogen-antagonist effects in others. Raloxifene is effective to prevent and treat postmenopausal vertebral osteoporosis, with reduction of spine fractures and, in post-hoc analyses, non-spine fractures in high-risk subjects.

Kathleen O Hagmeyer et al., (1999) The review the efficacy and safety of the selective estrogen receptor modulator raloxifene hydrochloride in the prevention of osteoporosis. A MEDLINE search (January 1966–May 1998) and unpublished data obtained from the manufacturer were used to identify relevant studies and review articles. Additionally, bibliographies of selected articles were reviewed. Only English-language articles on human studies were obtained. Literature was reviewed to evaluate the pharmacology, pharmacokinetics, therapeutic use, adverse effects, and drug interactions of raloxifene. Additional relevant citations were used in the introduction material and clinical controversy section.

Vaibhav Burhanpurkar., (2023) Osteoporosis, a systemic progressive disease, is responsible for significant morbidity and mortality in aging postmenopausal women. It is an important public health problem because of its significant complications, namely fractures of the proximal femur (hip), vertebrae (spine), distal forearm, proximal humerus, pelvis, and other skeletal sites. Compared with other osteoporotic fractures, hip fractures incur the greatest morbidity and direct medical costs for health services. There are now a variety of treatments available for the management of osteoporosis. Inhibitors of bone resorption, including calcium, vitamin Ds, bisphosphonates, calcitonins and gonadal steroids prevent bone loss or reduce fractures. Prevention of osteoporosis with identification of risk factors, careful examination and a few simple diagnostic tests during teen and early adult years is superior to treatment of older individuals. The purpose of this article is to provide a review of osteoporosis.

Ki Chan An., (2016) Selective estrogen receptor modulators (SERMs) are now being used as a treatment for breast cancer, osteoporosis and postmenopausal symptoms, as these drugs have features that can act as an estrogen agonist and an antagonist, depending on the target tissue.

After tamoxifen, raloxifene, lasofoxifene and bazedoxifene SERMs have been developed and used for treatment. The clinically decisive difference among these drugs (i.e., the key difference) is their endometrial safety. Compared to bisphosphonate drug formulations for osteoporosis, SERMs are to be used primarily in postmenopausal women of younger age and are particularly recommended if there is a family history of invasive breast cancer, as their use greatly reduces the incidence of this type of cancer in women. Among the above mentioned SERMs, raloxifene has been widely used in prevention and treatment of postmenopausal osteoporosis and vertebral compression fractures, and clinical studies are now underway to test the comparative advantages of raloxifene with those of bazedoxifene, a more recently developed SERM. Research on a number of adverse side effects of SERM agents is being performed to determine the long-term safety of this class of compounds for treatment of osteoporosis.

Miriam F. Delaney., (2005) During the perimenopause, both the quantity and quality of bone decline rapidly, resulting in a dramatic increase in the risk of fracture in postmenopausal women. Although many factors are known to be associated with osteoporotic fractures, measures to identify and treat women at risk are underused in clinical practice. Consequently, osteoporosis is frequently not detected until a fracture occurs. Identification of postmenopausal women at high risk of fracture therefore is a priority and is especially important for women in early postmenopause who can benefit from early intervention to maintain or to increase bone mass and, thus, reduce the risk of fracture. Several pharmacologic agents, including the bisphosphonates (eg, alendronate, risedronate, and ibandronate) and the selective estrogen receptor modulator, raloxifene, have been shown to increase bone mass, to reduce fracture risk, and to have acceptable side-effect profiles. Women who have discontinued hormone therapy are in particular need of monitoring for fracture risk, in light of the accelerated bone loss and increased risk of fracture that occurs after withdrawal of estrogen treatment.

Irene Falsetti et al., (2022) Osteoporosis (OP) is a chronic disease that occurs when the balance between the processes of bone formation and resorption is lost. OP is characterized by a decrease in bone quality and an increased risk of fractures. In post-menopausal women, as a result of decreased estrogen levels, there is bone loss. Hormone replacement therapy was initially used for the management of OP in post-menopausal women but was soon abandoned due to the occurrence of significant side effects. This shifted research toward the development of a class of drugs called selective estrogen receptor modulators (SERMs). These drugs always act through estrogen receptors (ERs), but as agonists or antagonists depending on the tissue under consideration. In particular, SERMs at the level of bone tissue behave as agonists of ERs but, as they do not result in the occurrence of estrogen side effects, they are widely used in the therapy of post-menopausal OP. This review provides a brief summary of the characteristics of SERMs employed in the treatment of post-menopausal OP.

V. Chitra et al., (2020) Osteoporosis is a long-term systemic bone disease of developing relevance due to the change in current demography. If left untreated it even leads to the mortality of a person. Osteoporosis is becoming a major public health threat globally. Since the mechanism of this disease are still not fully understood and the treatment options are not satisfactorily involved, the research in osteoporosis was needed especially in an animal model. OVX rodents are commonly

used to study the osteoporosis model. Some aspects can only be labeled in larger models only. Among the larger animal model sheep is used and have been used for orthopedic implants. There is no perfect animal model for osteoporosis, the appropriateness of an animal model is not only defined regarding the similarity to the human physiology and disease itself. The pathogenesis of osteoporosis is understood by establishing the various animal model and the pre-clinical testing of the anti-resorptive drugs. The animal model is required to simulate the osteoporotic behaviour has been always different from that of the pharmacological testing. This review aims to have an idea on the current animal models in osteoporosis research and their assessment of bone mass and microarchitecture.

Farkhondeh Pouresmaeili et al., (2020) Osteoporosis is a bone disorder with remarkable changes in bone biologic material and consequent bone structural distraction, affecting millions of people around the world from different ethnic groups. Bone fragility is the worse outcome of the disease, which needs long term therapy and medical management, especially in the elderly. Many involved genes including environmental factors have been introduced as the disease risk factors so far, of which genes should be considered as effective early diagnosis biomarkers, especially for the individuals from high-risk families. In this review, a number of important criteria involved in osteoporosis are addressed and discussed.

Ermanno Bonucci et al., (2014) Osteoporosis is a very common skeletal disorder characterized by reduced bone mass and altered trabecular microarchitecture that leads to bone fragility and fractures. Such disease is due to alterations of the remodeling process that occurs in the basic multicellular units that are transitory cellular complexes including an osteoclastic phase (osteoclast activation and resorption of microscopic portions of bone), a reversion phase (osteoclast replacement by so-called postosteoclastic cells), and an osteoblastic phase (osteoblastic reconstruction of the resorbed bone matrix till the initial volume is regained). Bone remodeling is regulated by a number of systemic and local factors; among the former, besides physical activity and mechanical stresses, a primary role is played by hormones such as parathyroid hormone, vitamin D metabolites, estrogens, calcitonin, and glucocorticoids; among the latter, several growth factors (macrophage colony-stimulating factor, transforming growth factor b, platelet-derived growth factor, fibroblast growth factor 1, bone morphogenetic protein, and insulin-like growth factor 1), as well as the osteoprotegerin-receptor activator of nuclear factor- B ligand system and the sclerostin, play a primary function. The remodeling phases can be evaluated by static and dynamic histomorphometry. Their abnormalities may lead to several osteopathies, the most common of which is osteoporosis (above all senile and postmenopausal), a rather elusive disease chiefly due to its slow development. The use of animal models in its study is emphasized.

2.3 FUTURE SCOPE OF RESEARCH WORK

There is a large coverage of evidences that links the disorders of BMD with the long-term usage of anti-epileptic drugs intake in the patients who are suffering from epilepsy. Much data is seen from the patients which are treated with conventional anti-epileptic drugs. But now there is an urgent requirement for monitoring the effects of the new AEDs in the process of bone mineral metabolism.

As the patients who are having epilepsy are more attached to falls that is due to many reasons as increased frangibility of bones which can easily translate it into increased chances of skeletal fractures that causes high morbidity & mortality. It is further signified that an ageing population and increase in the chances of epilepsy is higher in elderly. The women with epilepsy are highly vulnerable to the disorders of bone health which results in the differences in hormonal & physiological parameters as compared with its male parts. The physicians that are testing the patients with epilepsy are required to get awaked with this problem.

It is also required that the potential of AEDs induced osteopenia and its prevention mainly in patients with many risk factors as post-menopausal women and elderly men. The good health practices should be given to the patient as to be more and more calcium along with Vitamin D, regularly weight bearing exercises, exposure of sunlight and avoid smoking and intake of alcohol.

CHAPTER 3

MATERIALS AND EXPERIMENTAL TECHNIQUES / METHODOLOGY

3.1 INTRODUCTION

The disease Osteoporosis is a major systemic disorder that affects many Caucasian women having diversed and multifactorial etiologies. A large variety of animal species that includes rodents, rabbits, dogs, primates are highly used as animal models of osteoporosis research. In all of these, the laboratory rats are the most preferred animals for their research study. The skeleton has also been studied properly. With this there are many limitations with its similarity as per human condition that can be overcome through complete knowledge of its particular traits or with other techniques. The usage of rats is under many experimental protocols that leads to bone mass by including hormonal interventions along with immobilizations and dietary manipulations.

There is a major requirement in further characterizing the known animal models for the condition of post-menopausal osteoporosis in better understanding of the pathogenesis of this disease that further investigates with the newer therapies along with evaluation of the prosthetic non-human primates, dogs, cats, rodents, rabbits and other laboratory animals with having advantages as well as disadvantages.

The model of sheep is a very helpful model for many reasons as they are docile, easy handling, housing, inexpensive, availability in large number, ovulate spontaneous, bone is large for the orthopaedic implants. Most of the animals' models have taken females over males for osteoporosis. Currently, an interest in developing a proper prosthetic device that could stimulate osteointegration into osteoporotic bone, axial bone, mandibular bone and others.

The augmentation of osteopenic lumbar vertebrae along with bioactive ceramics is a different area that requires testing in proper animal model. By using different animal models for the study of different facets of osteoporosis the reduces some of the difficulties that are linked with the disease study on humans that name time & behaviour variations among its test subjects. The new experimental drug therapies & orthopaedic implants can be checked on larger number of animals that are subjected to a level of experimentation control which is impossible in human clinical research.

3.2 MATERIALS

3.2.1 ANIMALS USED IN THE STUDY

Female Swiss albino mice (25-35 g) were employed; they were bred in the animal's house of Pranveer Singh Institute of Technology, Kalpi Road, Bhaunit, Kanpur 208020 having Registration No1273/PO/S/09/CPCSEA with the prior approval from Institutional Animal Ethical Committee (IAEC), bearing approval according to CPCSEA guidelines for carrying animal activity. Healthy female Swiss albino mice were used for activity. The animals were housed under standard condition as prescribed and had a proper approach to water and feed with the exclusion of food during the period. The animals were given a week to acclimate before the experiment began.

The animals were kept in a room at a constant temperature of 23.2 degrees Celsius, humidity of 55.15 percent, and a light-dark cycle of 12 hours. The cages were made of polypropylene. Mouse were given a pellet food and access to water at will. All tests were conducted during daylight hours (between 9:00am- 5:00 pm). The protocol and all procedures involving the use of animals in experiments were carried out in accordance with the university's regulations.

3.2.2 DRUGS

Phenytoin Sodium (PHT)	Unichem Pvt. Ltd., India
Sodium Valproate (SVP)	Unichem Pvt. Ltd., India
Levetiracetam (LTM)	Unichem Pvt. Ltd., India
Raloxifene (RLX)	Dr. Reddy's Laboratories Pvt. Ltd., India
Calcium and Vitamin D3 (CVD)	Cipla™, Cipla Ltd., India
Vitamin D3	Calcikind™, Mankind Pharma Ltd., India

Table 3.1: The list of various drugs used in the research study.

3.2.3 CHEMICALS AND REAGENTS

Bovine Serum Albumin	CDH, Mumbai
Chloramine-T	Sd Fine
Citric Acid	Sd Fine
Copper Sulphate	Sd Fine
Diethyl Ether	CDH, Mumbai
Folin Ciocalteau Phenol reagent	CDH, Mumbai
Formaldehyde AR	Sd Fine
Hydrochloric Acid	Sd Fine
Hydroxyproline (HxP)	Sd Fine
n-propanol	CDH, Mumbai
p-Dimethylaminobenzaldehyde	CDH, Mumbai
Perchloric acid	CDH, Mumbai
p-Nitrophenyl Phosphate	Sd Fine
Potassium Chloride	Sd Fine
Sodium Carbonate	Sd Fine
Sodium Citrate	CDH, Mumbai
Sodium Hydroxide	CDH, Mumbai
Sodium Potassium Tartrate	CDH, Mumbai
Triethanolamine	CDH, Mumbai

Table 3.2: The list of various chemicals & reagents used in the research study.

3.2.4 DIAGNOSTIC KITS

Alkaline Phosphate (ALP)	SPN Diagnostic, Surat, India
Calcium	Reckon Diagnostics Pvt. Ltd., Baroda, India
Creatinine	Reckon Diagnostics Pvt. Ltd., Baroda, India
Estradiol (E2) ELISA	Cusa Biotech, China
TGF-beta 3 ELISA	Cusa Biotech, China

Table 3.3: List of Various Diagnostic Kits used in the research study.

3.2.5 EQUIPMENT'S/APPARATUS

Cooling Centrifuge	Sigma, USA
DEXA	Hologic, USA
Electroconvulsometer	Techno, India
Electronic Balance	Samsung, Japan
ELISA Reader	Electronics Corporation of India Ld., Hyderabad
Homogenizer	Remi, Mumbai
HPLC	Waters, Japan
Incubator	Hicon, Grovers Enterprises, India
Laboratory Centrifuge	Remi, Mumbai
pH meter	Mettler, Switzerland
Refrigerator	Whirlpool, India
Ultra-low temperature freezer	New Brunswick Scientific, England
UV Spectrophotometer	PerkinElmer

Table 3.4: The list of various equipment's used in the study.

3.3 LAYOUT OF AN EXPERIMENT

3.3.1 CHOICE OF DOSES

Human and animal doses were used to derive the following RLX, PHT, SVP, LTM, CVD, and CVDD (CVD + VD) doses**.**

Drugs	**Animal Dose /Human Dose**	**Corresponding Mice Dose**	**Dose Selected**
PHT	35*	35	35
SVPL	100*	100	100
SVPH	300*	300	300
LTML	50#	70	100
LTMH	150#	210	200
RLX	120€	15	15
CVD	Ca(1000mg) + VD (500 IU/12.5 µg)	Ca(130mg/kg) +VD (65 IU/1.63 µg)	130mg/kg + 65 IU/1.63 µg
VD	2000IU/50µg (2000-500=1500 IU/37.5 µg	260 IU/6.5µg=195 IU/4.88 µg	195 IU/4.88 µg

*Mice Dose (already used), # Rat dose (already used) €Human Dose

PHT=Phenytoin, SVPL= Sodium Valproate Low Dose, SVPH=Sodium Valproate High Dose, LTML=Levetiracetam low dose, TLMH=Levetiracetam high dose

Table 3.5- Various dosage adjustments for research study based on human or animal dosages

3.3.2 DRUGS THAT ARE TAKEN ORALLY

All three ingredients (PHT, SVP, and LTM) were dissolved in DE. Carboxymethylcellulose aqueous solution (1%), in which RLX, CVD, and CVDD were suspended (CMC). All medications were taken orally.

3.4 METHODS OF EXPERIMENT

SPECIFICALLY, THE FOLLOWING STEPS MADE UP THE EXPERIMENTAL PLAN:

3.4.1 MODELING BONE LOSS DUE TO AEDS (PHT, SVP, AND LTM)

For conducting the study, we have taken sixty animals that were divided into six groups of ten. As per four months the usage of anti-epileptic medications were provided once daily by mouth intake as PHT is given 35mg/kg, SVPL is given 100mg/kg, SVPH is given 300mg/kg and LTML is given 100mg/kg and LTMH is given 200mg/kg as given in Table 12. After the duration of 3 months of therapy and at plasma levels as per therapeutic range it was found that on previous standardization dose of PHT that is 35mg/kg induced bone loss in the mice femur. Then it was decided to give an extra month to see as if the lumber bones that changed.

Group No (n=10)	**Drug Treatment**	**Dose (mg/kg p.o.)**
1	Vehicle	1 ml/kg
2	PHT	35
3	SVPL	100
4	SVPH	300
5	LTML	100
6	LTMH	200

n = number of mice

Table 3.6: Antiepileptic drug-induced bone loss model treatment plan

Urine was collected to estimate urinary calcium and blood was taken under ether anesthesia to separate the serum and measure drug concentration four months after therapy started. Finally, the animals were killed so that femur and lumbar bone samples could be obtained for histological and biochemical examination.

3.4.2 PREVENTIVE THERAPY

There were twelve groups of ten animals where each is compromised with the experimental population of 120 animals. The oral intake of RLX, CVD or CVDD forwarded the intake of AEDs as PHT, SVP, LTM and at the time when the plasma concentration of the bone protects medications that reaches to Cmax. The drug PHT & SVP choses AEDs doses that are known to cause bone loss.

Group No. (n=10)	**Drug Treatment**	**Dose (mg/kg.p.o)**
1	Vehicle	1ml/kg
2	RLX	15
3	PHT	35
4	SVP	300
5	LTM	200
6	RLX+PHT	15+35
7	RLX+SVP	15+300
8	RLX+LTM	15+200
9	CVD+PHT	(130mg/kg+65 IU) +35
10	CVD+SVP	(130mg/kg+65 IU) +300
11	CVD+LTM	(130mg/kg+65 IU) +200
12	CVD	130mg/kg+65 IU

Table 3.7: Plan for preventative group treatment.

3.4.3 CARE FOR THERAPEUTIC PURPOSES

The animals (120 quantity) are used in the study that is divided into 12 groups f 10. Except for the groups, the animals were given AEDs (PHT, SVP, LTM) for a complete of 4 months. Then for an more month the animals were grouped as 3,4 and 11 that were given RLX or CVDD while in groups 1,2 & 12 were given only vehicle.

Group no.	Drug Treatment	Dose(mg/kg.p.o)
1	Vehicle	1ml/kg
2	RLX	15
3	PHT	35
4	SVP	300
5	LTM	200
6	PHT+RLX	35+15
7	SVP+RLX	300+15
8	LTM+RLX	200+15
9	PHT+CVDD	35+[(130mg/kg+65 IU) +(195 IU)]
10	SVP+CVDD	300+[(130mg/kg+65 IU) +(195 IU)]
11	LTM+CVDD	200+[(130mg/kg+65 IU) +(195 IU)]
12	CVDD	(130mg/kg+65 IU) +(195 IU)]

Table 3.8: Therapeutic treatment plan.

Then after that urine collection is done for over 24 hrs after the conclusion of both the preventive as well as therapeutic treatments are used in determining the total amount of calcium un the urine. The serum-estradiol level is also determined after an overnight fasting period from the mice (E2). Fur then mice were given euthanasia as soon as blood was removed and then their femur & lumbar spine (L2-L4) were removed for the histopathology estimation, biochemical estimation for example, alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRAP) & hydroxyproline (HxP). bone mineral density (BMD) was analysed using DEXA scan.

3.4.4 ELECTROCONVULSIVE GROUP

For determining the impact of raloxifene on the efficiency of AEDs in a model of seizure control, the mice were separated into 12 groups of 10 animals each and challenged with electro-shock followed by chronic therapy as seen in table;

Group no. (n=10)	Drug Treatment	Dose(mg/kg.p.o)
1	Vehicle	1 ml/kg
2	RLX	15
3	PHT	35
4	SVP	300
5	LTM	200
6	PHT+RLX	35+15
7	SVP+RLX	300+15
8	LTM+RLX	200+15
9	PHT+CVDD	35+(130mg/kg+65 IU)
10	SVP+CVDD	300+(130mg/kg+65 IU)
11	LTM+CVDD	200+(130mg/kg+65 IU)
12	CVDD	(130mg/kg+65 IU)

Table 3.9: Electroconvulsive therapy group's planned treatment regimen

3.4.5 METHODOLOGY

3.4.5.1 ELECTROSHOCK INDUCED SEIZURES

By the use of electro-convulsiometer the mice are reached for inducing convulsions (ECT Unit, Italy). The electrodes of ear-clip are used in delivering an alternative current that maximum voltage stimulation voltage of 500 V with a fixed current supply of 21 mA for 0.2 seconds. The highest range of motion of the hind limbs was determined as tonic extension that is defined as an extension of 180 degrees from body-axis plane (Borowicz et al., 2007).

3.4.5.2 BONE MINERAL DENSITY AND BONE MINERAL CONTENT ANALYSIS BY DEXA

There is removal of soft tissue from left femur and lumbar vertebrae (L2-L4) before they were frozen at -80 degree Celsius. After deforesting for around 30 minutes the bones were scanned by the help of DEXA for BMD analysis.

3.4.5.3 SAMPLE PREPARATION

3.4.5.3.1 BONE TISSUE SAMPLE PREPARATION

The muscles & other tissues were cut away from the lumbar vertebrae (L2-L4) and the bones were extracted. Then separate weighing of bones was done, further homogenized in triethanolamine buffer solution of 10mM volume with pH 7.5. After the process of homogenization, then 1.5 hrs at 4 degrees Celsius with continuous stirring the mixture was centrifuged. The bone extracts were processed two times for ensuring enough quantities by measuring the alkaline phosphatase & tartrate resistant acid phosphatase activity. Then for determining the amount of hydroxyproline the insoluble pellets were heated to 105 degrees Celsius in 6N Hcl for 24 hrs.

3.4.5.3.2 SERUM PREPARATION

Each animal's blood sample (about 2.5 times the volume needed for usage) was incubated at room temperature, upright, for 45 minutes. The samples underwent a 10-minute centrifugation at 3,000 rpm following the clot's retraction. The resulting serum was frozen in liquid nitrogen for later use.

3.4.5.3.3 URINE COLLECTION

The urine was collected for 24 hours and then frozen at -20 degrees Celsius in a sterile flask.

3.4.5.3.4 PARAMETERS ASSESSED

S. No	Parameters	Sample
1.	Maximal Electroshock Induced Seizures (MES) for Pharmacological Evaluation	
2.	Histopathology & Bone Mineral Density (BMD) for bone studies	Femur and Lumbar
3.	Alkaline Phosphatase (ALP), Tartrate Resistant Acid Phosphatase (TRAP), Hydroxyproline (HxP), Calcium	Lumar (L2-L4), Urine

	(Ca-U) as Bone Turnover Markers	
4.	Estradiol (E2), Transforming Growth Factor – beta 3 as related Markers	Serum, Lumbar (L2-L4)

Table 3.10: Various Parameters assessment for bones.

3.4.6 POSSIBLE APPROXIMATIONS AND HOW TO USE THEM:

ESTIMATION OF HYDROXYPROLINE

Method: Bergman and Loxley, 1963

Principle:

To get the "pink colour," free hydroxyproline (HxP) is oxidized with chloramine T to pyrrole, and then pyrrole is reacted with p dimethylaminobenzaldehyde. The resulting colour is then analyzed using spectrophotometry at a wavelength of 562 nm.

Conc.(µg/ml)	HxP standard (ml)	Double distilled water (ml)	Sample taken (ml)	HCL (ml)	n-propanol(ml)	Oxidant (ml)	Ehrlich's reagent (ml)
25	0.05	0.95	0.1	0.4	1.0	0.5	1.0
50	0.10	0.90	0.1	0.4	1.0	0.5	1.0
75	0.15	0.85	0.1	0.4	1.0	0.5	1.0
100	0.20	0.80	0.1	0.4	1.0	0.5	1.0
200	0.40	0.60	0.1	0.4	1.0	0.5	1.0
300	0.60	0.40	0.1	0.4	1.0	0.5	1.0

Table 3.11: Procedure for determination of hydroxyproline with sample, HCL, n-propanol, Oxidant and Ehrlich's reagent.

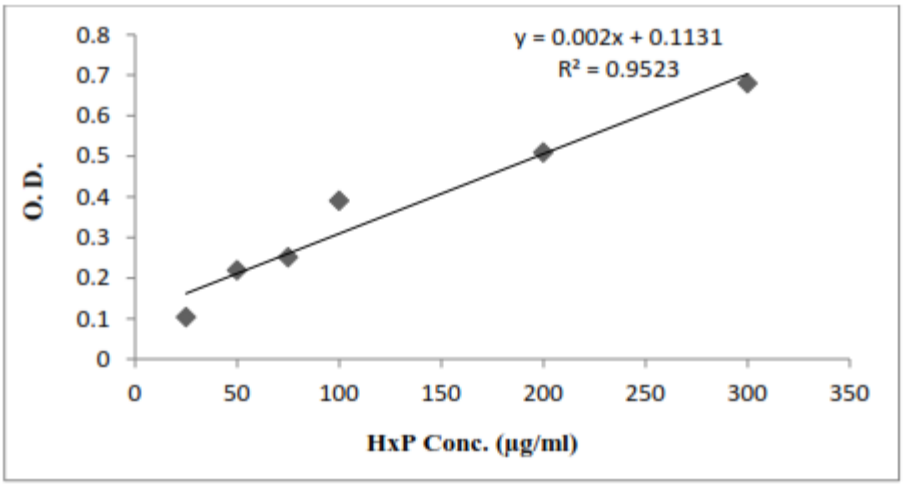

Figure 3.1: Calibration curve for hydroxyproline

Procedure

The HCl hydrolysate was resuspended in 1 mL of 1 mM HCl and then transferred to Eppendorf tubes for centrifugation at 3,000 rpm for 5 minutes at room temperature. The following were added to each test tube: (1) 100 L of supernatant, (2) 400 L of 1mM HCl, (3) 1 mL of n-propanol, and (4) 500 L of oxidant solution. For 4 minutes, the tubes were kept at room temperature. Then, 1.0 ml of newly made Ehrlich's reagent was added to each tube. The tubes were then placed in a water bath incubation at 60°C for 21 minutes. After 60 minutes at room temperature, the tubes were chilled in an ice bath. The optical density was measured at 562 nm using a blank solution (500 L of 1mM HCl added in place of sample).

ESTIMATION OF SERUM ESTRADIOL LEVEL

Serum levels of estradiol (E2) were measured across all groups using an ELISA kit marketed by Cusa Biotech with the catalogue number CSB-E05109m.

PRINCIPLE OF THE ASSAY

This analysis was conducted using the competitive inhibition enzyme immunoassay technique. The microtiter plate that comes with this kit already has the goat anti-rabbit antibody coated on it. Either standards or samples are added to the corresponding microtiter plate wells after the addition of the antibody against E2 and Horseradish Peroxidase (HRP) conjugated E2. A competitive inhibitory reaction between HRP-tagged E2 and unlabeled E2 is triggered by an antibody. Following the addition of a substrate solution to each well, the color shift will directly correspond to the amount of E2 present in the sample. A color's development is halted and then measured to determine its intensity.

REAGENTS USED FOR THE STUDY;

S. No	Reagent	Quantity
1	Assay Plate	96wells
2	Standard	3ml
3	Antibody	6ml
4	HRP-conjugate	6ml
5	Wash buffer (20 × concentrate)	15ml
6	Substrate A	7ml
7	Substrate B	7ml
8	Stop Solution	7ml

Table 3.12: Various laboratory reagents for measuring level of estradiol in human serum.

3.4.7 STATISTICAL ANALYSIS

The data was analyzed using GraphPad Prism 3.0 (GraphPad Software; SanDiego, CA, USA). Average Standard Error of the Mean was the statistical distribution used to describe the data. Multiple comparison tests, first with Tukey and then with Kramer, were used to examine differences between groups using data collected using ANOVA. In all instances, significance was assumed at a P value of 0.05 or lower.

3.4.8 SUMMARY

The Methodology comprises of Animals that is Female Swiss Albino Mice with further Anti-Epileptic Drugs along with Chemicals, Diagnostic kits and other equipment's were used during the research study. Further, the experimentation includes a layout for the research study which comprises the choice of drugs, its route of administration. Then the methods of experiment were summarized according to the studies conducted. The Methodology of the experimentation includes induction of disease, analysis with the process, preparation of the sample, collection of urine and assessing of parameters. At last, all the data collected was further collected and then verified by the statical analysis of the data.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

Analyzing the effects of three different AEDs on bone changes and creating a model of bone loss caused by AEDs in female mice.

4.2 RESULTS

4.2.1 HISTOPATHOLOGICAL ANALYSIS

The values were shown as Mean SEM. The HLE stands for "hind limb extension," "Control" is "Vehicle" (Carboxymethylcellulose, 1ml/kg), "PHT" is "Phenytoin" (35 mg/kg), "SVP" is "Sodium Valproate" (300 mg/kg), "LTM" is "Levetiracetam" (200 mg/kg), "RLX" is "Raloxifene" (15 mg/kg), and "CVD". All of the drugs were taken by oral route at the same time for four months of duration.

4.2.2 DEXA INVESTIGATIONS

4.2.2.1 BONE MINERAL DENSITY (BMD) & EPILEPSY

Over the course of a person's life, their bones undergo continual remodeling as an organ that is metabolically active. Osteocytes, osteoblasts, and osteoclasts are the main cell types found in bones. Collagenous and non-collagenous proteins are produced by osteoblasts to build unmineralized bone matrix.

These cells have an alkaline phosphatase isoform that can be used as a surrogate indicator of osteoblastic activity. They produce and handle type 1 collagen. The cells called osteoblasts, which are integrated into the calcified bone matrix, are in charge of the nutrition exchange. The monocyte-macrophage lineage gives rise to osteoclasts, which aid in the breakdown of bone matrix (Fitzpatrick LA., 2004). Through the ongoing remodeling process of bone resorption and production, bone plays a crucial role in the metabolism of calcium and phosphorus. A normal skeleton's cortical and trabecular bones go through a full remodeling cycle in roughly 100 and 200 days, respectively.

DEXA measurements revealed that the femur and lumbar vertebrae (L2-L4) have altered bone mineral density. After four months of treatment with 35 mg/kg of PHT and 300 mg/kg of SVP, the mice's lumbar vertebrae and femurs had considerably less bone mineral density (BMD) than the control group. SVP (100 mg/kg), however, had no effect on lumbar spine BMD or femoral bone

mineral density (BMD). After four months of treatment, there was no difference in bone mineral density (BMD) between the 100 mg/kg and 200 mg/kg LTM groups.

4.2.2.2 BONE MINERAL CONCENTRATION (BMC)

Chronic therapy with PHT (35 mg/kg) decreased BMC significantly over four months. However, SVP (100, 300 mg/kg) and LTM (100, 200 mg/kg) did not affect BMC.

4.2.2.3 CONCENTRATIONS OF ANTIEPILEPTIC DRUGS IN PLASMA

Blood drug concentrations were found to be within the therapeutic range (10-20 g/ml) after four months of PHT (35 mg/kg). The serum medication concentrations at 100 and 300 mg/kg SVP were within the human therapeutic range (50-100 ng/ml). With 200 mg/kg, serum concentrations of LTM were within the therapeutic range (12-46 g/ml), but not with 100 mg/kg.

4.2.3 SIGNS OF BONE RESORPTION

Bone development markers (alkaline phosphatase, ALP) and bone turnover markers (tartrate-resistant acid phosphatase (TRAP), hydroxylysine (HxP), and urine calcium (U-Ca) in the lumbar vertebrae of mice were modified after four months of AED therapy.

4.2.3.1 ALKALINE PHOSPHATASE (ALP)

Bone ALP activity was decreased in the lumbar vertebrae when treated with PHT (35 mg/kg p.o.) or SVP (300 mg/kg p.o.) but not when treated with SVP (100 mg/kg p.o.) or LTM (100 and 200 mg/kg p.o.). The significance level for this differentiation was 0.001.

4.2.3.2 TARTRATE RESISTANT ACID PHOSPHATASE (TRAP)

Bone TRAP activity was significantly increased (p0.001) in mice treated with PHT (35 mg/kg) and SVP (300 mg/kg) compared to controls (p0.05 with SVP 100mg/kg) but was not significantly altered in mice treated with LTM (100, 200 mg/kg).

4.2.3.3 HYDROXYPROLINE (HXP)

The referring (HxP) concentration of the vertebral column of treated animals with PHT (35 mg/kg) (p0.001) and SVP (100, 300 mg/kg) (p0.05, p0.001) was lower when compared to the controls. This was the case for both treatments. The amount of HxP did not alter when the animals were given LTM at 100 or 200 mg/kg doses.

4.2.3.4 URINARY CALCIUM (U-CA)

Four months after starting treatment with PHT (35 mg/kg), SVP (100 and 300 mg/kg), and LTM (200 mg/kg, but not 100 mg/kg), U-Ca was considerably greater than before treatment.

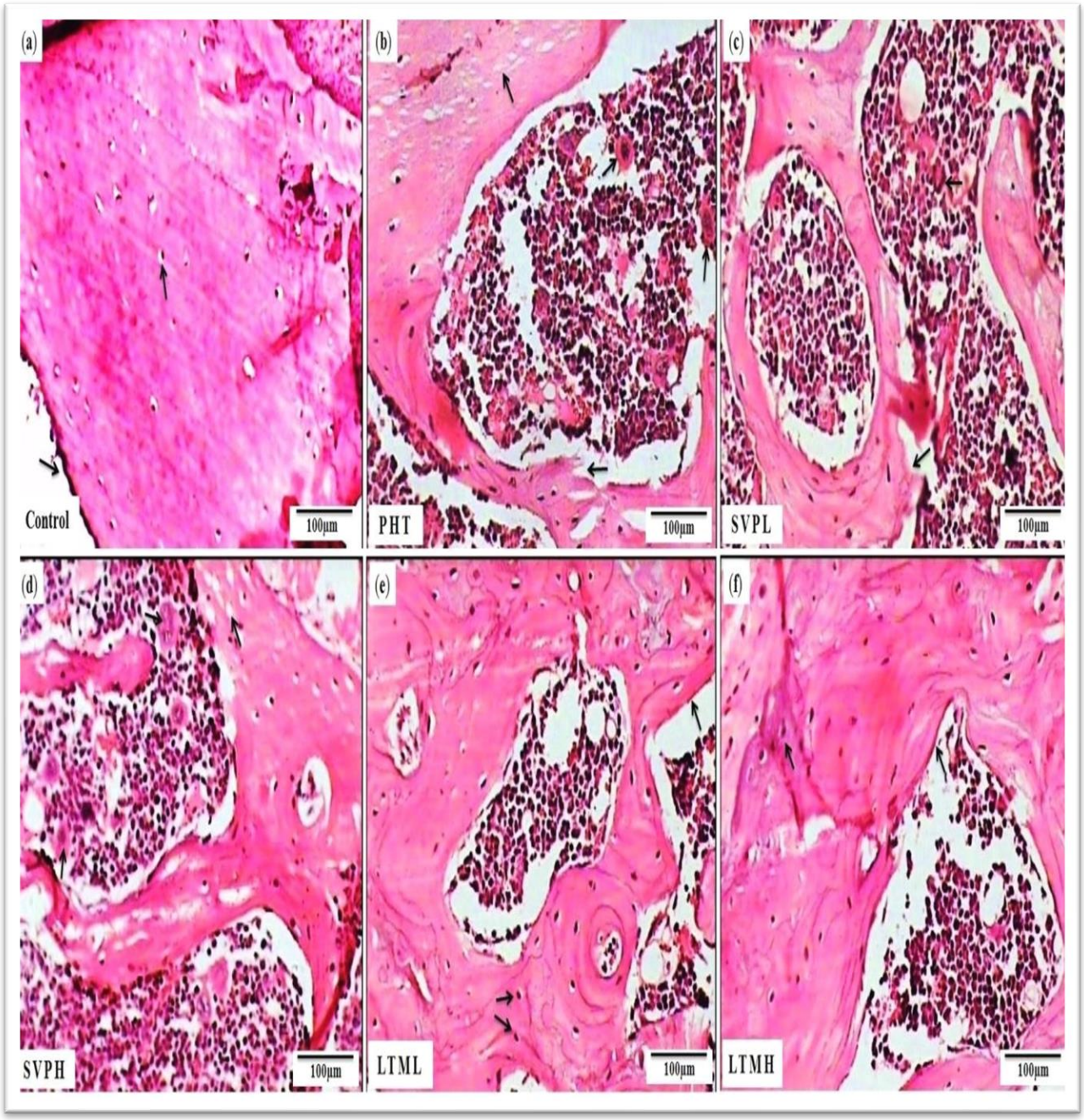

Figure 4.1: Mice femoral histopathology after 16 weeks of chronic treatment with antiepileptic drugs (PHT, SVP, and LTM)

Osteoclastic activity, rarefaction of bone matrix, and a ruffled border were observed in the femurs of animals treated with PHT for four months in comparison to controls. A higher osteoclastic activity, ruffled boundary, and rarefaction of the bone matrix was seen in response to SVP at both doses. Neither dose of LTM (fig. 13e, 13f) resulted in any noticeable alterations, in contrast to the effects of PHT and SVP (magnification, 40X).

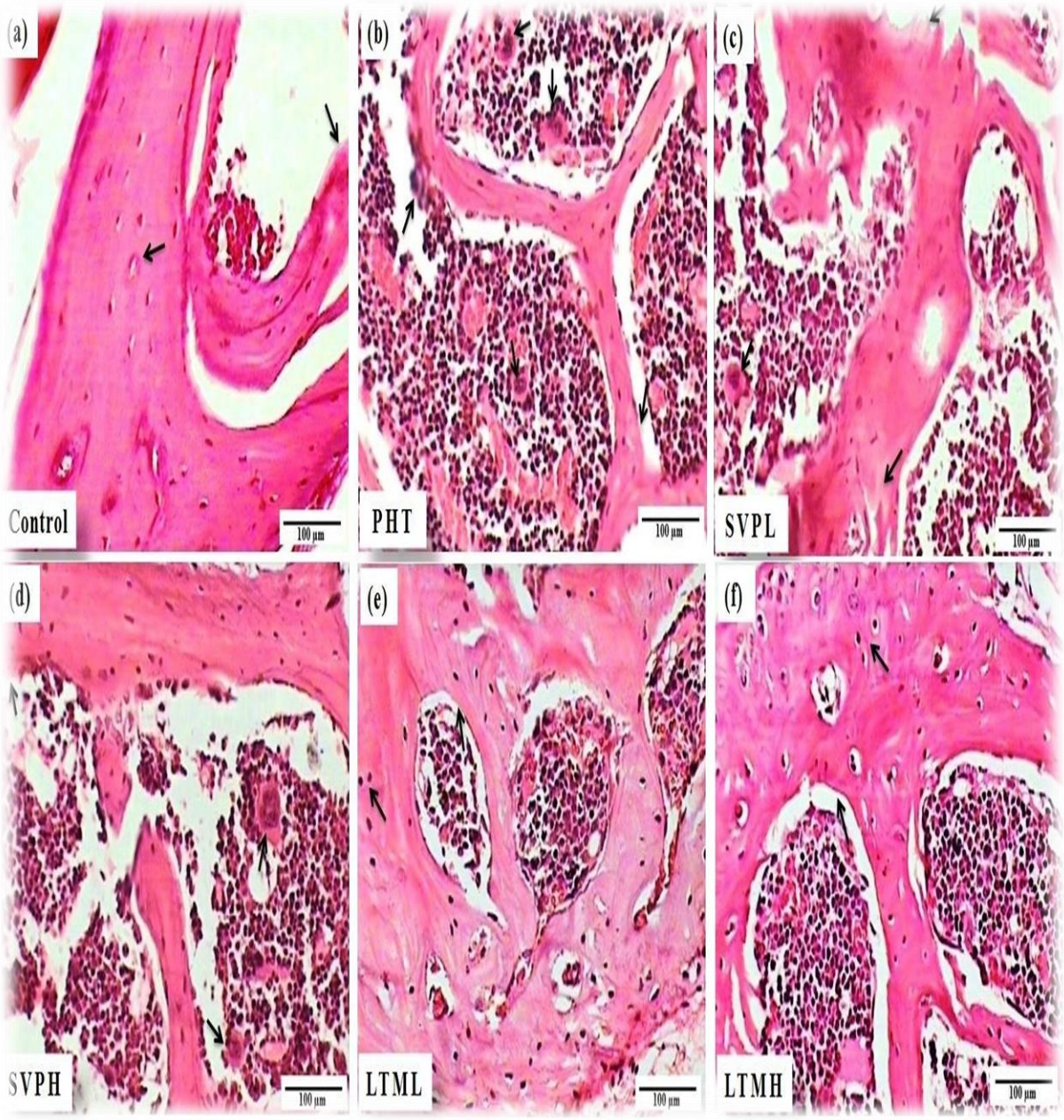

Figure 4.2: Histological changes in the lumbar vertebrae of mice subjected to chronic antiepileptic medication (PHT, SVP, and LTM) administration for 16 weeks

Osteoclastic activity, rarefaction of bone matrix, and a ruffled border were observed in L4 lumbar vertebrae (a-f) after four months of PHT treatment, in contrast to controls. Increased osteoclastic activity, a ruffled border, and rarefaction of the bone matrix were seen after exposure to SVP at both doses. Neither dose of LTM resulted in any alterations, in contrast to what was seen with PHT and SVP (magnification, 40X).

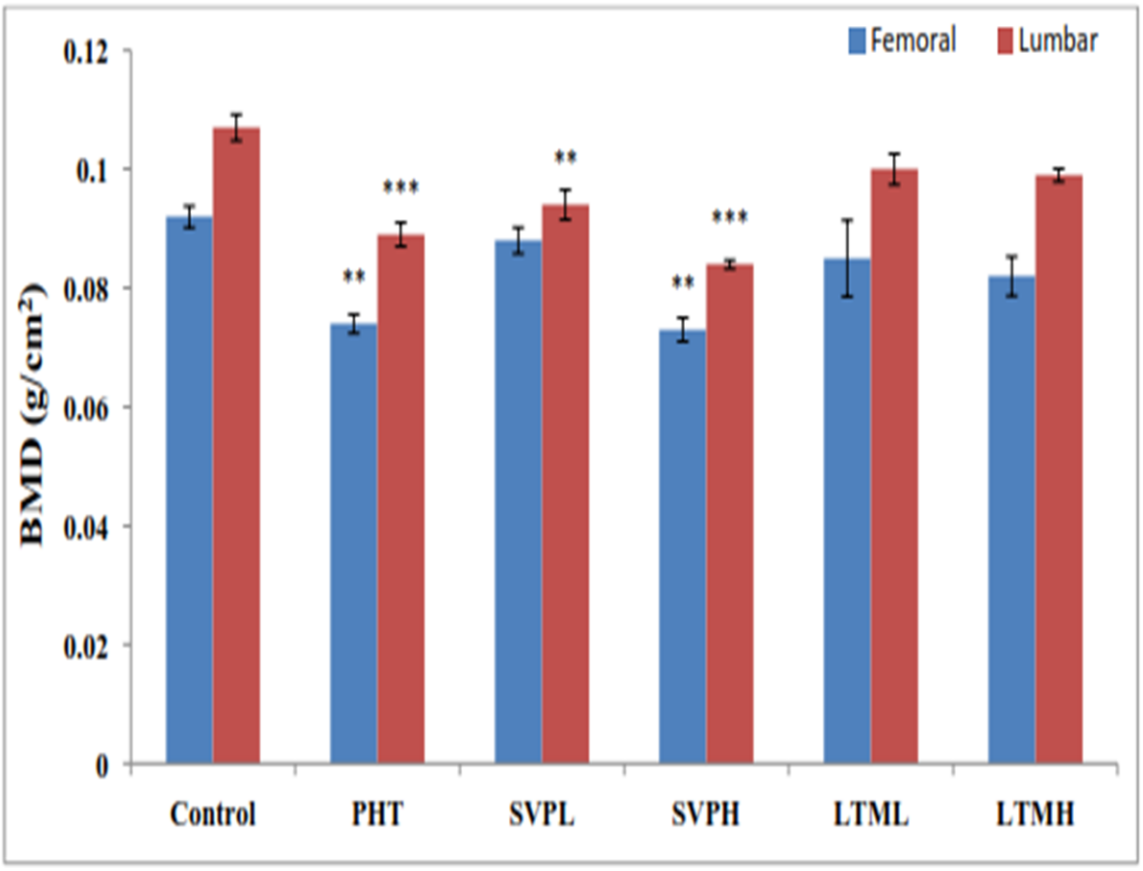

Figure 4.3: Bone mineral density (BMD) in mice's femur and lumbar vertebrae was analysed after exposure to phenytoin, sodium valproate, and levetiracetam

In each group, there are a total of six creatures. The data was consistently shown using a mean and standard error of the mean. There were no injections or surgical procedures involved in the treatment; it was all done through pills taken orally over four months. The doses of phenytoin, sodium valproate, levetiracetam that were used in the study ranged from 35 mg/kg to 300 mg/kg, in comparison to their respective controls, p0.05, p0.01, and p0.001.

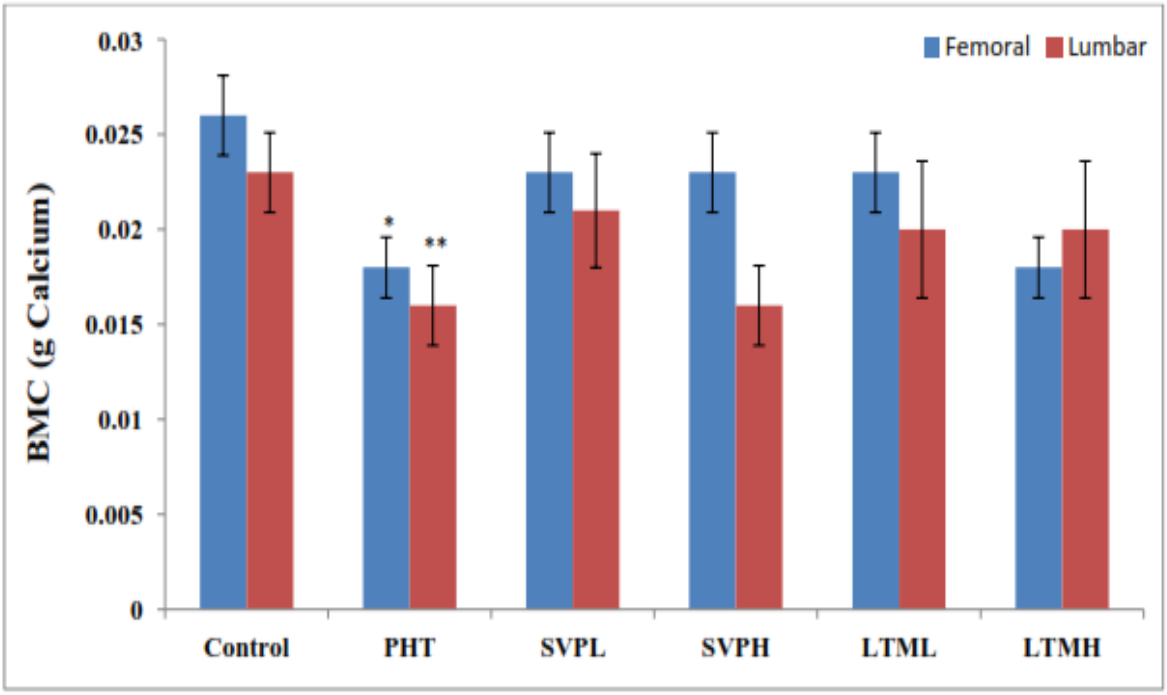

Figure 4.4: Bone mineral content (BMC) of mouse femurs and lumbar vertebrae after treatment with phenytoin, sodium valproate, and levetiracetam.

Within each category, there are a total of six unique animals. All values were expressed as the mean SEM. All drugs were orally taken over four months. Levetiracetam (100 mg/kg), Sodium Valproate (300 mg/kg), Levetiracetam (200 mg/kg), and Phenytoin (35 mg/kg) were compared. *p0.05, **p0.01 significantly different from the matching control.

Groups	**Control**	**PHT**	**SVP**		**LTM**	
			Low Dose	**High Dose**	**Low Dose**	**High Dose**
Weight (gm)	29.2±0.59	27.4±1.08	27±0.59	26.3±0.96	28.9±0.76	27.9±0.67
Dose(mg/kg)	-	35	100	300	100	200
Plasma drug Conc.(µg/ml)	-	18.46±0.71	51.26±1.1	98.92±1.59	10.82±1.14	42.28±1.23

Table 4.1: Plasma levels of antiepileptic medicines (PHT, SVP, and LTM) after 16 weeks of treatment.

All values were presented as mean SEM and are shown for clarity: PHT, phenytoin (35 mg/kg); SVP, sodium valproate (100 mg/kg and 300 mg/kg); LTM, levetiracetam (100 mg/kg and 200 mg/kg).

S. No	Groups	ALP (µmol of PNP liberated/µg of protein)	TRAP (µmol of PNP liberated/µg of protein)	HxP (µmol of PNP liberated/µg of protein)	U-Ca (mg Ca/mg of Creatinine)
1	Control	12.98±0.85	0.53±0.02	155.96±2.93	0.066±0.005
2	PHT	4.83±0.44	1.21±0.06	103.28±1.45	0.119±0.004
3	SVPL	10.71±0.50	0.92±0.07	142.06±1.71	0.117±0.009
4	SVPH	6.57±0.39	1.17±0.12	122.64±3.45	0.149±0.012
5	LTML	10.93±0.60	0.83±0.05	145.3±3.74	0.077±0.006
6	LTMH	10.57±0.39	0.85±0.06	147.42±4.00	0.11±0.006

Table 4.2 - Effects of various chronic therapy with phenytoin, sodium valproate, and lamotrigine on biochemical markers of bone metabolism in mouse lumbar vertebrae

The average standard deviations from the mean (SEM) for each category are shown in the table below. Treatment with the full pharmacological regimen (Control = Carboxymethylcellulose, 1 ml/kg; PHT = Phenytoin, 35 mg/kg; SVPL = Sodium Valproate, 100 mg/kg; SVPH = Sodium Valproate, 300 mg/kg; LTML = Levetiracetam, 100 mg/kg; LTMH = Levetiracetam, 200 mg/kg) was given orally over four months. There are statistically significant differences between Groups 2 and 3 (Test) and Group 1 (Control) at the 0.05, 0.01, and 0.001 levels, respectively, when compared using ANOVA and Tukey-test Kramer's.

4.2.4 REVERSING AND PREVENTING BONE LOSS FROM PHT AND SVP WITH RALOXIFENE

4.2.4.1 Impact on Research Using DEXA Scan

Density of bone tissue (BMD)

The results of DEXA scans revealing changes in bone mineral density (BMD) in the lumbar spine and femur, respectively. Four months after treatment, PHT (35 mg/kg) and SVP (100 and 300 mg/kg) significantly decreased bone mineral density (BMD) in the lumbar vertebrae and femurs of PHT- and SVP-treated mice compared to controls. When RLX was stopped, bone mineral density (BMD) in the femur and lumbar spine returned to its pre-PHT and post-SVP values (Table 25 & 26). Although CVD prevented further loss of BMD, it was not as effective as RLX (p=0.01) or RLX + RLX (p=0.001) in restoring the lumbar vertebrae after PHT and SVP treatment or in the femur after SVP treatment, in restoring lost BMD. Similar outcomes were seen when RLX and CVDD (treatment) were given one month following PHT and SVP.

BONE MINERAL CONTENT (BMC)

The outcomes of a DEXA scan of the lumbar spine and femur to determine the bone mineral density (BMD). Mice given PHT (35 mg/kg) and SVP (100 and 300 mg/kg) for four months had significantly lower bone mineral density (BMD) in the lumbar vertebrae and femurs compared to the control group. When RLX was inhibited, bone mineral density (BMD) in the femur and lumbar spine returned to pre-PHT/SVP values (Table 25 & 26). Compared to RLX, CVD preventive treatment was significantly less effective in restoring bone mineral density (BMD) in the lumbar vertebrae after PHT and SVP and in the femur after SVP treatment (p0.01) (p0.001). One month after injection, RLX and CVDD (treatment) yielded results equivalent to PHT and SVP.

4.2.4.2 CHANGES IN BONE TURNOVER BIOMARKERS

ALKALINE PHOSPHATASE

Bone alkaline phosphatase (ALP) activity in the lumbar vertebrae was decreased by PHT (35 mg/kg p.o.) and SVP (300 mg/kg p.o.) considerably (p0.001). Although there was no significant difference (p>0.05) between the RLX, CVD, and CVDD groups and the control group concerning lumbar ALP activity, all three groups dramatically restored the AEDs-induced decline in bone ALP levels, with RLX being considerably more effective than CVD/CVDD.

4.2.4.3 ACID PHOSPHATASE WITH RESISTANCE TO TARTRATE (TRAP)

In the lumbar vertebrae of PHT (35 mg/kg) and SVP (300 mg/kg) treated mice, TRAP activity was considerably increased (p0.001). The lumbar TRAP activity was not significantly altered by adding RLX or CVD/CVDD, and both successfully reduced the high TRAP levels in the preventative and treatment groups.

4.2.4.4 HYDROXYPROLINE (HXP)

In comparison to controls, the administration of either PHT (35 mg/kg) or SVP (300 mg/kg) significantly decreased the amount of hydroxyproline (HxP) in the lumbar vertebra. Higher levels of HxP were seen in RLX-treated groups compared to PHT- and SVP-treated groups for CVD and CVDD. When used separately, these therapies did not significantly alter lumbar HxP levels.

4.2.4.5 URINARY CALCIUM (U-CA)

PHT (35 mg/kg) and SVP (300 mg/kg) medication significantly (p0.001) enhanced urinary calcium excretion compared to the control group (U-Ca). A substantial reduction in urinary Ca 2+ excretion was seen after therapeutic and preventative treatment of RLX (p0.001). CVD/CVDD yielded identical results to RLX, albeit with fewer severe consequences (p0.05).

Treatment	Groups	BMD		BMC	
		Femur	Lumbar Vertebrae	Femur	Lumbar Vertebrae
Therapeutic	Control (Veh.)	0.092±0.0018	0.107±0.0022	0.026±0.0021	0.023±0.0021
	PHT	0.074±0.0016	0.088±0.0009	0.018±0.0016	0.016±0.021
	PHT+RLX	0.095±0.0028	0.102±0.0027	0.033±0.0021	0.026±0.0021
	PHT+CVDD	0.098±0.0034	0.099±0.0005	0.026±0.0021	0.030±0.000
	RLX	0.098±0.002	0.111±0.0010	0.03±0.000	0.033±0.002
	CVDD	0.101±0.0022	0.104±0.0033	0.03±0.000	0.030±0.000
Preventive	PHT	0.074±0.0016	0.089±0.0020	0.018±0.0016	0.016±0.0021
	PHT+RLX	0.098±0.0018	0.102±0.0011	0.03±0.000	0.04±0.000
	PHT+CVDD	0.092±0.0026	0.1±0.0020	0.023±0.0021	0.023±0.0021
	RLX	0.099±0.0005	0.108±0.0024	0.033±0.0021	0.03±0.000

	CVDD	0.096±0.0049	0.103±0.0013	0.026±0.0021	0.026±0.0021

Table 4.3: Changes in bone mineral density (BMD) and bone mineral concentration (BMC) in mice femur and lumbar vertebrae induced by Phenytoin (PHT) and prevented by raloxifene (RLX).

Mean standard deviations are shown in this table. Bone Mineral Density (BMD); Bone Mineral Content (BMC); VEH, Vehicle; Carboxymethylcellulose; 1ml/kg); PHT, Phenytoin; RLX, Raloxifene; Calcium Vitamin D (130 mg/kg + 65 IU); Calcium Vitamin D (130 mg/kg + VD [(130 mg/kg + 65 IU) + (195 IU)]; PHT was an oral tablet regimen that lasted for four months. RLX and CVD were administered simultaneously for prevention to maximize their effectiveness and continued to be delivered for one full month after AED delivery for therapy. The statistical significance of our findings was determined as follows: Significance in the numbers: Statistical significance was determined as follows: *p0.05, **p0.01, ***p0.001 vs movement control; # p0.05, ##p0.01, ###p0.001 vs PHT.

Treatment	Groups	BMD		BMC	
		Femur	Lumbar	Femur	Lumbar
Therapeutic	Control (Veh.)	0.092±0.0018	0.107±0.0022	0.026±0.0021	0.023±0.0021
	SVP	0.073±0.0020***	0.084±0.0007***	0.023±0.0021	0.016±0.0021
	SVP+RLX	0.089±0.0029###	0.097±0.001###	0.026±0.0021	0.026±0.0021
	SVP+CVDD	0.093±0.0020###	0.095±0.0018**	0.026±0.0021	0.026±0.0021
	RLX	0.098±0.0020###	0.111±0.001###	0.03±0.000	0.033±0.0021
	CVDD	0.101±0.0022###	0.104±0.0033###	0.03±0.000	0.03±0.000
	SVP	0.073±0.002***	0.084±0.0007***	0.023±0.0021	0.016±0.0021

Preventive	SVP+RLX	0.098±0.0035$^{###}$	0.102±0.0012$^{###}$	0.03±0.0036	0.03±0.0036
	SVP+CVD	0.091±0.0023$^{##}$	0.096±0.0045**	0.023±0.0021	0.023±0.0021
	RLX	0.099±0.0005$^{###}$	0.108±0.0024$^{###}$	0.033±0.0021	0.03±0.00
	CVD	0.096±0.0049$^{###}$	0.103±0.0013$^{###}$	0.026±0.0021	0.026±0.0021

Table 4.4: Bone mineral density and bone mineral concentration variations in prophylactically and therapeutically raloxifene-treated mice at the femoral and lumbar vertebral levels (RLX)

The table shows the mean and standard deviation for these numbers. Bone Mineral Density (BMD) and Bone Mineral Content (BMC) were measured after administration of the following: Vehicle (Carboxymethylcellulose, 1 ml/kg); Sodium Valproate (300 mg/kg); Raloxifene (15 mg/kg); Calcium Vitamin D (130 mg/kg + 65 IU); CVD + VD [(130 mg/kg + 65 IU) + (195 IU)] (BMC). Patients took SVP orally for a total of four months. Both RLX and CVD were administered simultaneously for prophylaxis, and both were administered concurrently for therapy for one full month after the prescription of AEDs. To compare with the vehicle control group, *p0.05, **p0.01, and ***p0.001; to compare with the PHT group, #p0.05, ##p0.01, and ###p0.001.

Treatment	Groups	U-Ca^{2+} (mg Ca/mg of Cr)	ALP (µmol of PNP liberated/hr/µg of protein)	TRAP (µmol of PNP liberated/hr/µg of protein)	HxP (µg of HxP liberated/mg of Bone)
Therapeutic	Control (Veh.)	0.066±0.005***	15.16±1.06***	0.53±0.029	155.96±2.93
	PHT	0.134±0.005***	4.83±0.44***	1.21±0.065	103.28±1.45
	PHT+RLX	0.069±0.003$^{###}$	12.87±1.24**	0.064±0.056	148.35±8.39
	PHT+CVDD	0.102±0.004$^{###}$	10.55±0.41*	0.075±0.076	139.64±8.54
	RLX	0.061±0.01$^{###}$	13.44±1.48	0.58±0.082	152.7±4.42
	CVDD	0.064±0.01	11.8±0.91	0.59±0.086	148.85±7.77
Preventive	PHT	0.121±0.003	5.83±0.50	0.95±0.046	115.28±1.71

	PHT+RLX	0.078±0.011	14.0±0.94	0.59±0.03	152.05±3.54
	PHT+CVD	12.09±1.61	12.09±1.61	0.63±0.052	149.38±3.35
	RLX	14.99±0.99	14.99±0.99	0.53±0.032	153.9±4.99
	CVD	13.01±0.77	13.01±0.77	0.54±0.048	151.62±4.11

Table 4.5: The effects of both prophylactic and therapeutic treatment with Raloxifene on biochemical changes caused by Phenytoin in the lumbar vertebrae of mice (RLX).

Each value in this table is accompanied by its standard deviation from the mean. BMD, Bone Mineral Density; BMC, Bone Trace Minerals; VEH, Vehicle; Carboxymethylcellulose; 1ml/kg); PHT, Phenytoin; RLX, Raloxifene; VD, Variable Dose; Calcium Vitamin D (130 mg/kg + 65 IU); Calcium Vitamin D (130 mg/kg + VD [(130 mg/kg + 65 IU) + (195 IU)]; PHT was an oral tablet regimen that lasted for four months. RLX and CVD were administered simultaneously for prevention to maximize their effectiveness and continued to be delivered for one full month after AED delivery for therapy. For comparisons to the driver assistance group, * P 0.05, ** P 0.01, and *** P 0.001; for comparisons to the PHT group, # P 0.05, ## P 0.01, and ### P 0.001.

Treatment	Groups/BTM	U-Ca^{2+} (mg Ca/mg of Cr)	ALP (µmol of PNP liberated/hr/µg of protein)	TRAP (µmol of PNP liberated/hr/µg of protein)	HxP (µg of HxP liberated/mg of Bone)
Therapeutic	Control (Veh.)	0.066±0.005	15.16±1.06	0.53±0.029	155.96±2.93
	SVP	$0.149±0.012^{***}$	$6.57±0.39^{***}$	$1.17±0.128^{***}$	$122.64±3.45^{***}$
	SVP+RLX	$0.077±0.004^{###}$	$12.66±0.40^{##}$	$0.60±0.021^{###}$	$147.21±2.72^{##}$
	SVP+CVDD	$0.103±0.006^{*}$	$11.61±1.00^{#}$	$0.69±0.033^{##}$	$142.94±1.74^{#}$
	RLX	$0.061±0.01^{***}$	$13.44±1.48^{###}$	$0.58±0.082^{###}$	$152.7±4.42^{***}$
	CVDD	$0.064±0.01^{###}$	$11.80±0.91^{##}$	0.59±0.086	$148.85±7.77^{##}$
Preventive	SVP	0.123±0.007	7.56±0.757	0.98±0.037	149.87±2.36
	SVP+RLX	0.064±0.007	13.57±0.263	0.56±0.055	146.45±2.31
	SVP+CVD	0.081±0.006	12.29±0.798	0.062±0.04	146.45±2.31
	RLX	0.04±0.012	14.99±0.999	0.53±0.032	153.9±4.99

	CVD	0.055±0.014	13.01±0.774	0.54±0.048	151.62±4.11

Table 4.6: Mice are subjected to biochemical changes in the lumbar vertebrae after being given sodium valproate (SVP), and the prophylactical effects of raloxifene (RLX) treatment.

Mean standard deviations are shown in this table. We evaluated the effects on BMD and BMC by administering the following: Calcium Vitamin D (130 mg/kg + 65 IU); Calcium Vitamin D (130 mg/kg + 195 IU); Vehicle (Carboxymethylcellulose, 1 ml/kg); Sodium Valproate (300 mg/kg); Raloxifene (15 mg/kg); (BMC). Patients took SVP orally for a total of four months. RLX and CVD were administered simultaneously for prevention to maximize their effectiveness and continued to be delivered for one full month after AED delivery for therapy. *** $p<0.001$ Significant data, as determined by SVP standards (.05 level of significance).

4.2.5 EFFECTS OF LONG-TERM AED TREATMENT ON SERUM ESTRADIOL LEVELS, COMPARISON WITH RLX AND CVD/CVDD, AND OTHER FACTORS

Serum estradiol (E2) ranges for each treatment group. Upon long-term administration of PHT (35 mg/kg) and SVP (300 mg/kg), serum estradiol levels in mice significantly lowered (p0.001). RLX was superior to CVD in raising serum estradiol levels in both the preventative and therapy settings. RLX and CVD/CVDD did not significantly alter serum estradiol levels when tested separately.

Free blood estradiol levels were lower in the LTM group after 16 weeks of treatment (200 mg/kg p.o.), but the variation was not statistically meaningful ($p>0.05$). Therefore, contrary to expectations, neither the preventive (RLX) nor the remedial (CVD) treatments were effective in reducing free serum estradiol levels in comparison to the AEDs group.

Groups							
Control	PHT	PHT+RLX	PHT+CVD	RLX	CVD	CVDD	PHT+CVDD
{Preventive} Estradiol {E2; pg /ml)							
66.31±4.85	22.49±1.22***	64.10±2.20$^{\#\#\#}$	61.85±6.28$^{\#\#}$	75.59±9.24$^{\#\#\#}$	64.74±8.90$^{\#\#\#}$	--	--
{Therapeutic} Estradiol {E2; pg /ml)							
66.31±4.85	21.65±3.85***	64.5±1.85$^{\#\#\#}$	--	64.89±2.55$^{\#\#\#}$	--	60.39±4.49$^{\#\#\#}$	53.48±4.21$^{\#\#\#}$

Table 4.7: Mice with elevated phenytoin-induced blood oestrogen levels treated with raloxifene and calcium-vitamin D for preventative and therapeutic purposes

The values in this table are presented as a mean, standard error of the mean. Calcium Vitamin D (130 mg/kg + 65 IU); Calcium Vitamin D and Vitamin D (130 mg/kg + 195 IU). Estradiol (E2), Phenytoin (35 mg/kg), Raloxifene (15 mg/kg), and a Vehicle (carboxymethylcellulose, 1 ml/kg) served as the controls. PHT treatment lasted four months and involved taking tablets orally. For prevention, RLX and CVD were given at the same time, and for therapy, RLX and CVD were provided for a full month after the administration of AEDs. *** p<0.001 Against a normative sample; ### p0.01; ### p0.001 when compared to PHT.

Groups							
Control	SVP	SVP+RLX	SVP+CVD	RLX	CVD	CVDD	SVP+CVDD
{Preventive}Estradiol (E2; pg/ml)							
66.31±4.85	21.65±3.22***	55.21±3.33##	47.96±1.96#	75.59±9.24#	64.74±8.90###	-	-
{Therapeutic}Estradiol (E2; pg/ml)							
66.31±4.85	21.20±2.54***	52.19±3.56###	-	64.89±2.55###	-	60.39±4.49###	50.69±1.01###

Table 4.8: Serum estradiol levels in mice were altered by sodium valproate, but pre-emptive and curative treatments with raloxifene and calcium-vitamin D prevented or reversed these effects.

Mean standard deviations are shown in this table. Vitamin D2 (VD) [(130 mg/kg + 65 IU) + (195 IU)], Raloxifene (15 mg/kg), and Vehicle (Carboxymethylcellulose, 1 ml/kg) for Sodium Valproate (300 mg/kg). Patients took SVP orally for a total of four months.

RLX and CVD were administered simultaneously for prevention to maximize their effectiveness and continued to be delivered for one full month after AED delivery for therapy. * p<0.05; *** p<0.001 Illustration with a motor vehicle. The following degrees of statistical significance is attained when compared to the SVP norm: # p0.05; ## p0.01; ### p0.001.

Groups							
Control	LTM	LTM+RLX	LTM+CVD	RLX	CVD	CVDD	LTM+ CVDD

{Preventive}Estradiol (E2; pg/ml)							
66.31±4.85	52.38±4.34	56.38±1.71	55.13±7.45	75.59±9.24	64.74±8.9	-	-
{Therapeutic}Estradiol (E2; pg/ml)							
66.31±4.85	48.72±4.35	53.81±4.81	-	64.89±2.55	-	60.39±4.49	56.23± 3.36

Table 4.9: The impact of calcium-vitamin D and raloxifene on preventing and treating levetiracetam-induced alterations in blood estradiol levels in mice

4.2.6 COMPARISON OF THE IMPACT OF RLX AND CVD/CVDD ON LUMBAR TRANSFORMING GROWTH FACTOR 3 (TGF- 3) TO THE IMPACT OF PROLONGED TREATMENT WITH AEDS

Examine the differences in lumbar TGF-3 concentrations across different groups. TGF-3 levels in the lumbar spine were significantly decreased in mice given PHT (35 mg/kg) and SVP (300 mg/kg) on a long-term basis (p0.001). Preventative or curative RLX treatment prevented the decline in lumbar TGF-3 seen in response to PHT and SVP, and treatment with RLX restored pre-existing levels.

However, the drop in lumbar TGF-3 caused by PHT and SVP was only statistically significant in the SVP-treated group, and CVD and CVDD somewhat recovered this decrease. No improvement was seen when either RLX or CVD/CVDD was used alone.

The level of lumbar TGF-3 was significantly decreased after 16 weeks of chronic LTM (200 mg/kg p.o.) treatment compared to the control group. TGF-3 levels were significantly increased following both preventative (RLX) and curative (CVD) treatment compared to the LTM group.

Mean standard deviations are shown in this table. Calcium Vitamin D (130 mg/kg + 65 IU) Control, Carboxymethylcellulose (1 ml/kg) Phenytoin (35.0 mg/kg) Raloxifene (15.0 mg/kg); PHT was an oral tablet regimen that lasted for four months. RLX and CVD were administered simultaneously.

Groups							
Control	PHT	PHT+RLX	PHT+CVD	RLX	CVD	CVDD	PHT+CVDD
{Preventive}TGF-beta 3 (pg/mg of bone)							
34.99±3.61	14.06±1.20***	24.15±0.65*	21.84±1.21	29.03±2.25###	25.84±2.71##	-	-
{Therapeutic} TGF-beta 3 (pg/mg of bone)							
34.99±3.61	11.37±1.26***	27.4±1.67*	-	28.6±2.41###	-	24.1±1.53	15.87±5.1**

Table 4.10: Mice with phenytoin-induced alterations in transforming growth factor-3 (TGF-3) in the lumbar bones were treated with raloxifene and calcium-vitamin D for prevention and treatment for prevention to maximize their effectiveness and continued to be delivered for one full month after AED delivery for therapy. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ Different from PHT, statistically significant data (at the 0.05 level).

Groups							
Control	SVP	SVP+RLX	SVP+CVD	RLX	CVD	CVDD	SVP+CVDD
{Preventive}TGF-beta 3 (pg/mg of bone)							
34.99±3.61	8.86±2.66***	23.95±0.65*	20.44±1.45##	29.03±2.25###	25.84±2.71##	-	-
{Therapeutic} TGF-beta 3 (pg/mg of bone)							
34.99±3.61	9.84±1.84***	22.06±1.42*	-	28.6±2.41###	-	24.1±1.53	20.78±3.3**

Table 4.11: Sodium valproate alters Transforming Growth Factor-3 (TGF-3) in mouse lumbar bones; raloxifene and calcium-vitamin D have a preventative and therapeutic effect.

The values in this table are presented as a mean, standard error of the mean. Sodium valproate (300 mg/kg), Carboxymethylcellulose (1 ml/kg), Vitamin D2 (VD) [(130 mg/kg + 65 IU) + (195 IU)], Raloxifene (15 mg/kg), and Vehicle (Carboxymethylcellulose, 1 ml/kg). People took oral SVP for a total of four months. The AEDs were prophylactic, while the RLX and CVD were used therapeutically for a full month after the initial AED treatment. * p<0.05; ** p<0.01; *** p<0.001 Statistically significant (at the.05 level) numbers: by the criteria of SVP.

Groups							
Control	SVP	SVP+RLX	SVP+CVD	RLX	CVD	CVDD	SVP+CVDD
{Preventive}TGF-beta 3 (pg/mg of bone)							
34.99±3.61	14.31±4.79***	22.62±4.85*	20.31±3.11##	29.03±2.25	25.84±2.71	-	-
{Therapeutic} TGF-beta 3 (pg/mg of bone)							
34.99±3.61	13.29±2.00***	24.07±2.84*	-	28.60±2.41##	-	24.1±1.53#	25.32±0.75**

Table 4.12: Transforming Growth Factor-3 (TGF-3) in mouse lumbar bones: effect of preventative and therapeutic raloxifene and calcium-vitamin D treatment

The values in this table are presented as a mean, standard error of the mean. Vehicle (Carboxymethylcellulose, 1 ml/kg); CVD, CVD + VD [(130 mg/kg) + (65 IU) + (195 IU)]; Calcium Vitamin D (130 mg/kg); Levetiracetam (200 mg/kg); Raloxifene (15 mg/kg); Patients were given oral LTM for four months. The AEDs were prophylactic, while the RLX and CVD were used therapeutically for a full month after the initial AED treatment. When compared to LTM, the significance levels are as follows: # p0.05; ##p0.01; * p0.05; ** p0.01; *** p0.001 for vehicle control.

4.2.7 LONG-TERM TREATMENT WITH RLX ALONE AND IN CONJUNCTION WITH AEDS FOR ELECTRIC SHOCK-INDUCED SEIZURES

Whenever PHT 35 mg/kg, SVP 300 mg/kg, and LTM 200 mg/kg were given prior to electroshock convulsions, tonic hind limb extension was completely abolished (HLE). Not only did combinations of AEDs and RLX not alter the tonic propagation phase of seizures, but they also yielded responses that were indistinguishable from those obtained with the administration of AEDs alone. Both AEDs' antiepileptic actions were unaffected by raloxifene. The duration of the effects and the time it took to reach a tonic state did not significantly differ across the groups.

S. no	Groups	Dose (mg/kg p.o)	Latency to HLE	Duration HLE	Number of animals showing protection against TLE	% Protection
1	Control	10 ml/kg	3.6±0.33	25.06±2.46	0/8	0
2	PHT	35	--	-	8/8	100
3	SVP	300	--	-	8/8	100
4	LTM	200	8.2±1.14	12.21±0.84	6/8	75
5	PHT+RLX	35+15	-	-	8/8	100
6	SVP+RLX	300+15	-	-	8/8	100
7	LTM+RLX	200+15	6.7±0.63	12.8±0.75	6/8	75
8	PHT+CVD	35+130+65 IU	-	-	8/8	100
9	SVP+CVD	300+(130+65 IU)	-	-	8/8	100
10	LTM+CVD	200+(130+65 IU)	9.7±0.05	11.92±0.46	7/8	87.5
11	RLX	15	7.2±0.87	13.36±3.40	0/8	0
12	CVD	(130+65 IU)	5.7±1.29	15.36±2.04	5/8	62.5

Table 4.13: A long-term study of raloxifene's effectiveness in preventing electroshock-induced seizures in mice, alone or in combination with AEDs.

4.2.8 Summary

The data was displayed using a mean and standard deviation of the mean plot. The placebo group received carboxymethylcellulose (1 ml/kg), the phenytoin group received 35 mg/kg, the sodium valproate group received 300 mg/kg, the levetiracetam group received 200 mg/kg, the raloxifene group received 15 mg/kg. The vitamin D group received 130 mg/kg + 65 IU. Everything in the medication mixture was consumed orally over four months.

After the complete results at last it is discussed that;

These results are the first experimental proof that raloxifene may help prevent and treat bone loss caused by PHT and SVP without affecting how well these drugs work to stop seizures (AED). This study adds to what was written by showing that AEDs have the same bad effects on bone in Swiss tension albino female mice. Also, the lack of bone effects after LTM diagnosis suggests that it may be better than PHT or SVP for women with epilepsy or people at risk for osteoporosis.

4.3 EVALUATION OF PHT, SVP, AND LTM ANTICONVULSANTS FOR SEIZURES IN RESPONSE TO RALOXIFENE TREATMENT

There are not any clear guidelines for how to deal with this problem yet, but Sheth and Harden (2007) say that all patients on long-term AED therapy should be screened for early detection of the therapy's possible effects on bone health and that CVD supplements should be given to all people who are likely to lose bone. Due to a lack of research on anti-osteoporosis drugs that can be prescribed with Androgen receptors, doctors do not know if anti-osteoporosis drugs could change how well AEDs work to protect the heart. Because of this, it is important to find out if anti-osteoporosis drugs increase the risk of convulsions. When giving an AED to a woman with epilepsy and osteoporosis, these things are very important to think about.

For over four months then it was looked that as how this raloxifene affected the people with seizures which are caused by electroshock, by its own as well as by the combination of PHT or SVP. Then we found that raloxifene caused seizures when electro-shock was used but it didn’t change HLE.

Further it was found RLX ability to survive after status epilepticus in rats fully. However, we did not find any protective effect of RLX in the ES model, even though we saw a non-statistically significant increase in the latency to HLE and a decrease in the length of HLE. To find out if RLX could help stop seizures, other seizure models, like threshold models, need to be used. When it was used with PHT, SVP, or LTM, the results were the same as when PHT, SVP, or LTM were used alone. This shows that long-term use of RLX does not change how well PHT, SVP, or LTM work to stop seizures.

4.3.1 BONE CHANGES CAUSED BY AEDS AND THE INFLUENCE OF RALOXIFENE/CVD/CVDD

After 4 months of treatment with PHT & SVP the bone changes were found in females. The Swiss albino mice, that means this mi ce can be taken for testing the possible drugs in case of treating bone loss. There is more clinical research required for conforming it. Still, LTM can be a safe option for women with epilepsy that are more prone to osteoporosis or with the risk factors.

4.3.2 HISTOPATHOLOGY

After three months of treatment, it was found that PHT caused histological changes in the femoral bones but not in the lumbar spine (Khanna et al., 2011).

In this case, we kept eyeing the lower back vertebrae for another month of therapy. In SVP, both doses increased the number of osteoclasts and the amount of bone matrix resorption, but the effects were stronger at 300 mg/kg. At any dose of LTM, there were no obvious changes in the structure of the bones of mice.

Measurements of bone mineral density (BMD) using the dual-energy X-ray absorptiometry (DEXA) method, the gold standard for diagnosing osteoporosis, verified the bone loss shown in histology. Both PHT and SVP significantly decreased BMD, which is in line with what has been seen in both preclinical and clinical studies of these AEDs. (Pack et al., 2008).

The effects of SVP on BMD seem to have been bigger in the long run than those on BMC. This study proved that SVP is bad for bone, similar to some but not all previous clinical findings. It was found that valproate had different effects on different kinds of animals. Two mouse strains (C3H/HeJ and Balb/c) were sensitive to valproate-caused bone loss, but one (A/J) was resistant. We added to the original study by showing that the Swiss albino mouse strain is just as susceptible to the skeletal problems that valproate can cause. The bone mineral density did not decrease after four years of treatment with LTM (100 or 200 mg/kg) (BMD). This makes sense since Nissen-Meyer and his colleagues found BMD in rats (Nissen-Meyer et al., 2007).

In the second study, however, it was found that LTM made the femoral neck weaker from a biomechanical point of view. Since most of the lumbar vertebrae (L2-L4) are trabecular bones, we looked into whether or not LTM changes these bones. Even though our research did not find any changes in the lumbar vertebrae, LTM may have different effects on the femoral neck and the lumbar areas.

There have been different reports, even in the clinical setting. After one year of treatment, it was seen that LTM monotherapy had no negative effects on bone strength and metabolism and no clear secondary effects on bone mass, quality (which is affected by bone micro-architecture, geometry, and bone matrix composition), or remodelling. When LTM was taken for two years, however, it

had the same effect on bone mineral density (BMD) as oxcarbazepine (OXC) (Beniczky et al., 2012).

The results of the previous study came from a single center, were based on a small number of patients, and did not consider fracture risk. This suggests that increasing the length of treatment or studying a larger group of people may have bad effects on bone after LTM is given. A recent study of young adults with epilepsy showed that those who switched from enzyme-inducing AEDs like PHT to LTM had higher bone mineral density (BMD) in their lumbar spine and femur (Phabphal et al., 2013).

There was great improvement in the bone mineral density of femur and lumbar vertebrae which was greatly improved by both preventative (RLX) and therapeutic (CVD) treatments after PHT and SVP. Vertebral fractures can be prevented by using RLX, which strengthens bones and increases bone mass.

The multiple outcomes of the raloxifene assessment (MORE) trial showed that a lower risk of vertebral fractures was linked to a higher lumbar bone mineral density (BMD). Our results are similar to those of the recent antiepileptic medication and osteoporosis prevention experiment (ADOPT) in epileptic males, which showed a positive effect of CVD supplementation on BMD in multiple places, including the lumbar spine (Lazzari et al., 2013). In this study, we looked at how raloxifene affects biochemical markers of bone turnover in trabecular bone (lumbar vertebrae), which is thought to be more sensitive to the drug.

4.3.3 BONE TURNOVER MARKERS

The results of bone mineral density (BMD) studies were backed up by those of bone resorption markers (BTMs), which give more information about the health of the skeleton in real time. The BTMs measure osteoblastic activity or how pro-collagen is broken down after it is released. Bone resorption signals better predict bone strength than bone production markers. In the early stages of growth, the glycoprotein alkaline phosphatase (ALP) is strongly expressed on the surface of osteoblasts (Christenson, 1997).

So, it is a very good way to measure the osteoblastic part of bone formation. With either PHT (35 mg/kg) or SVP (300 mg/kg), bone formation and osteoblastic activity were slowed down. A drop in vertebral ALP showed this. After treatment with RLX, CVD, or CVDD, ALP activity was higher in the lumbar spine (L2-L4). This shows that bone formation was better. Like other research, we found that the combination of ALP and RLX treatment was good. The treatment with RLX caused the mRNA levels of the derived cells signalling pathways Cbfa1/Runx2 and the two pro-collagen type I chain to go up (Taranata et al., 2002).

The increased ALP activity, which measures how much bone is being made, maybe because RLX has a tissue-specific estrogenic effect on bone, which promotes the development of osteoblasts

through the action of oestrogen. ALP activity was the same when 100 mg/kg of SVP or 100 or 200 mg/kg of LTM was given.

Tartrate-resistant acid phosphatase (TRAP), hydroxyproline (HSP), and urine calcium are all signs of bone resorption (U-Ca). Tartrate-resistant acid phosphatase (TRAP) is a highlighter kinase for osteoclastic bone resorption and one of the many lysosomal enzymes that osteoclasts make (Minkin, 1982). The osteoclasts remove the phosphate from the ruffled edge of the protein osteopontin, which is found in the bone matrix. This makes TRAP (Christenson, 1997).

It is a good biomarker to use when testing medicines to see if they can stop osteoclastogenesis from happening. It shows that LTM had no effect on TRAP activity but that TRAP activity was significantly (p0.001) higher in lumbar bones after continuous PHT and SVP therapy. This means that bones were breaking down quickly.

When RLX and CVD were used to treat this high TRAP activity, the activity decreased significantly. Following the same pattern as previous research, we found that RLX stopped bone resorption by lowering RANKL and raising OPG, stopping TRAP activity. This indicator of bone loss, also called HSP, comes from an amino acid that makes up about 13% of collagen (Delmas, 1993).

HSP is made when a proline in a chain of peptides is hydroxylated after translation. When there is a lot of HSP in urine and tissue, there is much osteoclastic activity and collagen breakdown (bone). Urine HSP has many problems as a clinical test, such as not being able to tell what kind of tissue is being tested and much metabolic breakdown. Most toxins, like HSP, are broken down in the liver and kidneys. Only 10–15% are passed out of the body in the urine (Swaminathan, 2001).

So, a more accurate way of measuring the amount of HSP in bone was found. The results of our study show that PHT and SVP treatments made the lumbar vertebrae of mice have more osteoclastic activity. As has been said, this may be because AEDs changed the collagen structure by making it cross-linking stronger. After RLX treatment, the amount of HSP in the bones went up in both the control and treatment groups (p0.001). The amount of HSP in the L2-L4 bones did not change with either dose of LTM (100 or 200 mg/kg). The changes in biochemistry were the same as we saw in bone mineral density (BMD) and histology.

Urinary calcium (U-Ca) has been used as a screening marker for abnormal bone turnover, even though it is not very sensitive. Postmenopausal women have a higher ratio of calcium to creatinine, which suggests that their bones break down and rebuild more quickly (Sachdeva et al., 2005).

After taking PHT and SVP, the ratio of calcium to creatinine in the urine (Ca/Cr) went up a lot (p0.001). With LTM treatment (higher dose), calcium in the urine went up, but not as much as with PHT and SVP. This means that the increase was insufficient to have a big effect on DXA. Hyperparathyroidism caused by a lack of vitamin D and Fanconi disease caused by SVP can cause an abnormally high U-Ca/Cr ratio. The abnormally high U-Ca/Cr ratio went down after treatment for prevention (RLX) and healing (CVD). The reversal may be caused by a rise in serum oestrogen, which makes calcium deposit on bone, or by an increase in TGF-3 expression, accompanied by its deposition on bone matrix and mineralization.

Osteoblastic differentiation is hindered due to downregulation of the Wnt pathway caused by sclerostin. Osteocytes are the source of sclerostin. Serum levels of sclerostin, which is secreted into the bloodstream, indicate that bone production is inhibited. In earlier research, serum sclerostin concentrations in breast cancer patients increased considerably from 29.5 pmol/L at baseline to 43.2 pmol/L following a 24-month course of anastrozole treatment. Nevertheless, a clinical trial is still required to employ sclerostin as a bone turnover biomarker.

Wnt signaling inhibitors DKK-1 and sclerostin are used as indicators for bone remodeling. Osteoblasts create DKK-1, which is then released into the bloodstream. The suppression of bone growth is reflected in the serum levels of DDK-1. DKK-1 levels in breast cancer patients receiving anastrozole treatment dropped from 34.3 pmol/L at baseline to 29.7 pmol/L at 24 months. The relationship between the BMD of the femoral neck and the whole hip has been demonstrated by DKK-1. To determine the clinical use of the regulator DKK-1 as a biomarker for osteoporosis assessment, further extensive research is required.

Most people agree that OPG is a soluble receptor that is released and is made by a variety of tissues and cell types, including osteoblasts. The function of OPG is to suppress osteoclastogenesis and act as a RANKL spoof receptor. Research on mice has shown that whereas OPG therapy of normal mice and overexpression of OPG in transgenic mouse models result in osteopetrosis, OPG knockout animals have severe osteoporosis.

Serum, plasma, EDTA, citrate, and heparin samples can all be used to measure OPG. Commercially available sandwich ELISA kits that use polyclonal detection and monoclonal capture antibodies are available for the analysis of OPG. More research is still needed to support the clinical utility of serum OPG as a biomarker for assessing the activity of bone disease.

Osteoblasts generate RANKL and OPG to control the osteoclasts' differentiation and maturation during the bone remodeling process. It has recently been shown that dextromethorphan inhibits RANKL-induced osteoclastogenesis and bone resorption by preventing NF-κB signaling from being activated in vitro. Oral dextromethorphan treatment ameliorates in vivo osteoporosis caused by ovariectomy.

Human serum levels of RANKL have been measured to evaluate the conditions in metabolic bone disorders. Even though serum RANKL has been explored for predicting fracture risk and assessing the response to osteoporosis treatment, there are still a lot of studies that need to be done before RANKL may be used clinically.

There are eleven isoforms of cathepsins, which belong to the cysteine protease family. Its strong kinin specificity is what distinguishes CTSK. The ruffled edge of actively resorbing osteoclasts is where CTSK is primarily expressed. To break down the proteins that make up the bone matrix, such as type 1 collagen, osteopontin, and osteonectin, osteoclasts produce CTSK into the bone resorption defect. Consequently, CTSK plays a significant role in the process of bone resorption. There is a substantial difference in the amount of CTSK between individuals with osteoporosis and controls. The outcome suggests that serum CTSK levels may be used as a possible biomarker for bone-mineral density and fracture prediction.

NTX-1 is typically measured by ELISA using a urine sample and is stable in urine for up to 24 hours at room temperature. As a bone resorption biomarker, urine NTX-1 has been utilized to evaluate postmenopausal women's fracture risk. Because urine NTX-1 does not change in response to meal intake and does not produce blood withdrawal, it is the biomarker of choice for practical use when compared to serum CTX-1.

Type 1 collagen telopeptides are well studied and utilized as indicators for bone resorption, such as carboxy-terminal crosslinked (CTX-1) and amino-terminal crosslinked (NTX-1). The breakdown of collagen releases both CTX-1 and NTX-1. Using a monoclonal antibody against an octapeptide sequence (EKAHD-β-GGR) in the α-1 (I) chain of the β-isoform, ELISA is used to assess CTX-1. According to a recent study, CTX-1 is a sensitive and specific biomarker of bone resorption that can be used to quickly determine how well postmenopausal osteoporosis treatment with bisphosphonates is working.

During bone resorption, osteoclasts often produce TRAP 5b. Therefore, for osteoclast activity and numbers, TRAP 5b is the reference. Once in the bloodstream, the hydrolyzed TRAP 5b is processed by the liver and eliminated through the urine. Immunoassays are a particular means of detecting TRAP 5b in serum. According to a previous study, patients with breast cancer with limited or substantial bone metastases can be identified using serum TRAP 5b. Moreover, serum TRAP 5b has been used to track how well alendronate therapy is working. After a thorough analysis, the TRAP 5b bone resorption biomarker was found to have good specificity and high sensitivity when compared to other bone biomarkers.

Phosphorylated glycoprotein OP is expressed by bone cells, activated T-lymphocytes, macrophages, specialized epithelial cells, and altered cells. According to a recent study, women with over-expression of OP exhibit lower resistance to postmenopausal osteoporosis than do women with normal levels of OP. In order to assess the effectiveness of intermittent parathyroid hormone treatment for menopausal osteoporosis, the levels of plasma OP may be utilized as a biomarker.

The apparent molecular weight of BSP, a phosphorylated glycoprotein, ranges from 60 to 80 kDa. Mineralized tissues, including bone, dentin, cementum, and calcified cartilage, contain BSP. Approximately 8% of all the non-collagenous proteins discovered in bone and cementum are formed by BSP, which is a significant part of the extracellular matrix of bone. Osteoblasts, odontoblasts, and osteoclasts produce BSP. Consequently, BSP is thought to play a significant role in cell-matrix adhesion processes as well as the promotion of osteoclast-mediated bone resorption. Immunoassays have been established by numerous research to measure BSP in serum.
PYD collagen crosslink is created when fibrillar collagens mature extracellularly and is discharged into the bloodstream when mature collagens degrade. According to earlier research, HPLC studies demonstrate the PYD's long-term chemical stability in both the free and conjugated forms. PYD, however, is present in blood vessels, ligaments, bone, and cartilage. In contrast to DPD, PYD is a non-specific bone resorption biomarker.

DPD is a chemical that forms crosslinks between individual collagen peptides to mechanically stabilize collagen. The crosslinked collagens are hydrolyzed by proteases during the resorption of bone, releasing DPD into the bloodstream for excretion in urine. The dentin and bone contain the

majority of DPD. DPD is therefore employed as a particular biomarker for bone resorption. Because DPD is discharged in the urine in two forms, free (40%) and peptide-bound (60%), preanalytical hydrolysis and extraction were performed on the DPD prior to HPLC analysis in previous work. The peptide-bound form is converted into free form for the HPLC measurement in order to increase accuracy.

An amino acid called HYL is produced when lysine undergoes a post-translational hydroxy modification. Galactosyl hydroxylysine (GHYL) and glucosyl galactosyl-hydroxylysine (GGHYL) are the two forms of HYL [41]. When collagen breaks down, GHYL and GGHYL are both released into the bloodstream. Because GHYL is solely produced by bone resorption, it is a more specific biomarker for bone. Conversely, GGHYL is present in the skin and the C1Q complement molecule. As a result, GHYL is thought to be a superior bone resorption biomarker than HYP and GGHYL. Previous research has used the concentration of GHYL in urine excretion to assess the risk of fracture in postmenopausal osteoporotic women who have not experienced fragility fractures and in women who have experienced fragility fractures.

An amino acid called HYP is produced when proline is post-translationally hydroxylated. Roughly 12–14% of the amino acids in mature collagen are found in HYP. About 90% of the HYP is released during the breakdown of bone collagen, and the liver is where the majority of the HYP's metabolism occurs after that. When comparing postmenopausal osteoporosis ladies to postmenopausal non-osteoporosis women, the level of HYP in their urine has dramatically increased. The rise in urine HYP suggests that osteoporotic women have higher levels of collagen type I degradation from the bone matrix.

P1CP is a single protein with a molecular weight of 115 kDa that has post-translationally added mannose-rich carbohydrate side chains. PICP has a brief serum half-life of 7-8 minutes because it is removed by liver endothelial cells through the mannose receptor. According to a recent study, the P1CP in serum is employed as a biomarker of bone growth to assess the impact of female sex hormones and nandrolone decanoate. Compared to women who had a forearm fracture (116 ± 27 ng/mL), the mean first PICP concentration in the former group (97 ± 23 ng/mL) was significantly lower.

Procollagen type 1 in bone develops into type 1 collagen, which is present in the organic bone matrix (> 90%). Fibroblasts and osteoblasts synthesis procollagen type 1. N- and C-terminal extensions of procollagen type 1 are eliminated during the conversion of procollagen to collagen by certain proteases. P1CP and P1NP, which are present in procollagen type 1, are then conjugated onto the bone matrix. One particular indication of type 1 collagen deposition is the P1NP bone formation biomarker.

Known by several names, including bone gamma-carboxyglutamic acid-containing protein, osteocalcin is a 49-amino acid protein. Mature osteoblasts, odontoblasts, and hypertrophic chondrocytes produce OC. Furthermore, making up around 2% of the total protein in the human body, OC is the most prevalent non-collagenous protein in bone. Osteoblast-produced OC is crucial for bone mineralization, metabolic control, and calcium ion balance. It has been shown that when osteoporosis patients are receiving treatment with bone-

forming medications, there is a strong correlation between the level of serum OC and the rise in BMD.

About half of all ALP in serum with normal liver function in people is made from bone. Because their main difference is posttranslational glycosylation, the ALP isoenzymes derived from bone are challenging to differentiate from those derived from liver. Numerous techniques, including agarose electrophoresis, heat and chemical inactivation, wheat germ agglutinin precipitation, wheat germ agglutinin-high performance liquid chromatography, immunoradiometric test, and enzyme immunoassay, have been used to measure different aspects of BALP.

The bloodstream contains the enzyme alkaline phosphatase. The human body's proteins can be broken down by the various ALP types. The liver produces the majority of ALPs, whereas the kidneys, intestines, and bones all create some ALPs. The amount of alkaline phosphatase enzyme in the blood is measured in order to assess the total ALP. Total ALP measurement is frequently a standard component of blood testing and just requires a straightforward blood collection. People's typical levels of ALP vary depending on their age, gender, blood type, and stage of pregnancy. ALP levels in the blood that are unusual usually point to a problem with the liver, gall bladder, or bones. One way to diagnose bone disorders like rickets is via the ALP test.

4.3.4 MODULATION SERUM ESTRADIOL AND LUMBAR TGF-3 BY RALOXIFENE/CYCLOPHOSPHAMIDE/DOXORUBICIN (AED)

When mice ate PHT and SVP, the amount of E2 in their blood dropped significantly. Previous research shows that PHT or SVP treatment lowers serum estradiol levels in both male and female rats. We found this about serum estradiol (Svedberg et al., 2002; Heinicke et al., 1984).

The action of PHT on sex hormone-binding globulin may have contributed to oestrogen insufficiency by lowering testosterone and other adrenal androgens that are aromatized into oestrogen. Still, polycystic ovarian syndrome or direct suppression of estradiol release after long-term therapy may be to blame for the lower levels of estradiol found in the blood after SVP. PHT and SVP have also been found to block aromatase by 50%. This keeps testosterone from turning into estradiol, lowering estradiol levels (Jacobsen et al., 2008).

Even though LTM stopped the release of estradiol when forskolin was present, it did not affect the production of estradiol on its own, and long-term LTM therapy did not lower serum estradiol levels. RLX and CVD/CVDD worked to bring back levels of estradiol that had been low before. By binding to tissue-specific oestrogen receptors, RLX makes it easier for oestrogen to protect bone. It does this by entering the nucleus and boosting gene transcription by bringing in multiple co-activators and co-repressors (Miki et al., 2009).

The I.4 and I.3 promoters can be used to control how much oestrogen is made by human osteoblasts. It has also been shown that brain glioma cells turn the aromatase promoter. It makes sense that having enough vitamin D would have the opposite effect on aromatase expression, as having too little vitamin D raises aromatase expression and, in turn, raises estradiol levels.

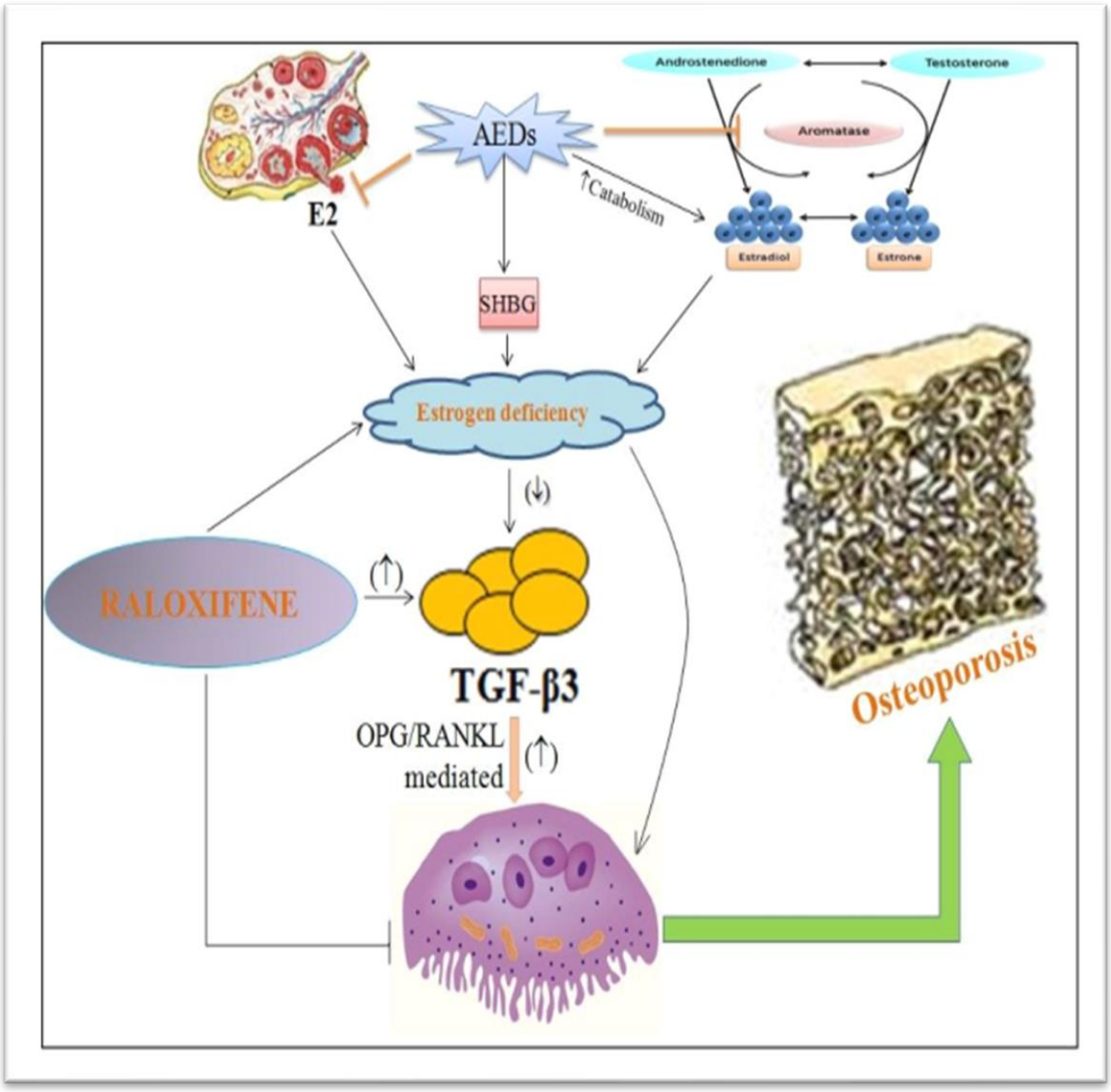

Figure 4.7: Scenario showing a possible series of events that might have happened after PHT and SVP caused bone loss and RLX stopped it.

AEDs (Sodium valproate, SVP, and Phenytoin, PHT) cause oestrogen insufficiency by either blocking aromatase complex and stopping the body from making oestrogen from C19 steroids or speeding up the breakdown of estradiol (which raises SHBG level) (thereby increasing SHBG level). The lack of oestrogen can lower TGF-3 and cause bone loss directly. It can also cause bone loss indirectly by shutting down antioxidant pathways and making more reactive oxygen species, which promotes osteoclastogenesis.

In this study, bone loss may have been caused by the expression of receptor activator of nuclear factor kappa B (RANKL) leads to osteoclastogenesis or a decrease in osteoprotegerin (OPG) on the surface of osteoblast cells (after estradiol was taken away). Raloxifene partially protects bones through direct estrogenic action and higher levels of oestrogen. It also partially protects bones through increased TGF- 3 expressions in bone, which helps osteoblasts grow and kills osteoclasts.

A transforming growth factor (TGF) protein controls how cells divide and what they will become in most living things. It is made in large amounts in response to things that make osteoclastic bone resorption happen, and there is a lot of it in the bone matrix. Studies have shown that oestrogen deprivation and ovariectomy lower the amount of TGF3 in rat bones (Finkelman et al., 1992).

We found that the TGF-3 levels in the lumbar bones of mice went down after PHT and SVP, which fits with this theory. Because TGF 3 is known to increase bone production by osteoblasts and reduce osteoclast activity by making osteoclasts commit suicide, it is possible that changes in BMD and BTMs caused the bone loss. These results suggest that decreased TGF-3 (after estradiol was taken away) may have caused bone loss by either increasing RANKL expression, which promotes osteoclastogenesis or decreasing osteoprotegerin (OPG) on the surface of osteoblast cells. Also, bones with less vitamin D have less TGF- than bones with more vitamin D. This means that vitamin D deficiency after PHT may reduce TGF-beta even more.

Our study showed that chronic LTM did not change the estradiol levels, but it did lower the levels of TGF-3 in the lumbar area. This finding goes against the idea that low estradiol levels cause a drop in lumbar TGF-3, at least for LTM. However, a previous study found that TIEG1 gene expression was downregulated in the hippocampus. This suggests that the decrease in lumbar TGF-3 may be due to its ability to stop expression. The drop in TGF-3 content caused by AEDs was reversed by RLX therapy. This is likely because it had an antagonistic effect on the TGF-3 promoter or because the estradiol level went up. Increased expression of the transforming growth factor-beta 3 (TGF-3) gene in rat bone has been suggested as a possible way to keep bones healthy. TGF-3 levels also increased with CVD/CVDD therapy, but not as much as with RLX.

This work adds to the growing body of evidence that suggests changes in estrogen-mediated TGF 3 contents may explain the bone loss caused by PHT and SVP. RLX, a selective oestrogen receptor modulator with estrogenic activity on bone and a TGF-agonistic effect, can reverse the changes these AEDs have made to the bones. Our results look good, but more research needs to be done before we can say what happens when RLX is added to an AED regimen over time.

CHAPTER 5
CONCLUSIONS & FUTURE SCOPE

5.1 CONCLUSION

People who take antiepileptic drugs can have several unwanted side effects, such as bone problems (AEDs). But there isn't much known about how anti-osteoporosis therapy affects bone loss caused by AEDs. We think a lack of estrogen and progesterone after treatment with an AED could be bad for bones. AEDs stop the aromatase enzyme from working in humans. This makes the microsomes break down more oestrogen. When there isn't enough oestrogen, there is less deposition of the bone matrix nutrients transforming growth factor-3 (TGF-3), which stops bones from breaking down. To learn more about how estradiol and transforming growth factor 3 affect the effects of AEDs or Raloxifene on bone, we compared the impact of these drugs on bone in mice given extra calcium and vitamin D3 (CVD). The effects of Raloxifene on seizure activity and the ability of AEDs to control epilepsy were also looked at.

Therefore, the following goals and objectives guided the development of this study:

For comparing the effects of phenytoin, sodium valproate & levetiracetam on bone changes in female mice.

The research was conducted to see if Raloxifene may counteract the changes in bone mineral density and bone turnover indicators caused by AEDs.

This study aims to examine Raloxifene's influence on the AED-inducing properties of electroshock-induced convulsions.

This study focused on determining whether oestrogen and transforming growth factor beta 3 (TGF3) mediate the effects of AEDs and Raloxifene on bone.

For four months, the effects of PHT (35 mg/kg, orally), SVP (100 mg/kg, orally), and LTM (100 mg/kg, orally) on the bones of female Swiss strain mice were studied. AED serum concentrations were put into therapeutic ranges that make sense from a medical point of view. The amounts of complementary vitamin D and calcium (CVD/CVDD) and RLX were based on clinical dose conversions for humans.

For the first four months of therapy with AEDs, preventive treatment (RLX or CVD) was given along with treatment (RLX or CVDD), which started one month later. Histology of the femur and lumbar spine, as well as Dual-energy X-ray densitometry for bone mineral density and osseous mineral content, were used to check for changes in the bones (DEXA). Also, the amount of calcium in the urine was measured, and the lumbar vertebrae were looked at for signs of bone resorption, such as enzymatic activity (ALP), tartrate conferring resistance acid phosphatase (TRAP), and

hydroxylysine (HxP). Also measured were the stages of TGF3 in the lumbar area and serum estradiol.

This investigation's key takeaways are summed up as follows:

5.2 TO COMPARE THE EFFECTS OF PHENYTOIN, SODIUM VALPROATE, AND LEVETIRACETAM ON BONE CHANGES IN FEMALE MICE

Mice administered 35 mg/kg of PHT and 300 mg/kg of SVP for four months revealed evidence of bone loss in the femur and long lumbar bones. These findings are supported by alterations in BTMs in the lumbar bones, including decreased alkaline phosphatase activity (a marker of bone formation), increased TRAP activity, decreased HSP concentration, and increased U-Ca excretion. In femoral and lumbar bone histological examinations, rarefaction of the bone matrix, a ruffled border, and the development of osteoclasts were all indicative of bone loss.

All of these changes happened at doses that put the drug concentration in the blood plasma far below what is safe and effective for humans.

When phenytoin and sodium valproate were given to Swiss albino female mice, their bones became less mineralized.

Histopathological examination, bone mineral density (BMD), bone mineral content (BMC), and bone turnover markers in the lumbar bones were all the same after four months of treatment with LTM (100 mg/kg and 200 mg/kg p.o.).

When bad effects on the bone from AEDs are considered, LTM may be better than PHT and SVP for women with epilepsy who are genetically programmed to or have a risk factor for osteoporosis.

5.3 RESEARCH WAS CONDUCTED TO SEE IF RALOXIFENE MAY COUNTERACT THE CHANGES IN BONE MINERAL DENSITY AND BONE TURNOVER INDICATORS CAUSED BY AEDS

With RLX (15 mg/kg p.o.) therapy, either for prevention or treatment, the decrease in BMD and BMC caused by PHT and SVP was reversed in a big way. CVD/CVDD also stopped BMD from being lost because of PHT and SVP, but not as well as RLX.

RLX is better than CVD/CVDD at stopping or treating bone loss caused by AEDs. It can also stop or treat osseous loss caused by PHT and SVP.

Alkaline phosphatase (ALP) behavior in the lumbar vertebrae was greatly reduced (p0.001) after treatment with PHT (35 mg/kg p.o.) and SVP (300 mg/kg p.o.). Even though there was no great disparity between the RLX, CVD, and CVDD organizations and the control subjects regarding

lumbar ALP activity, all three groups reversed the AEDs-caused drop in bone ALP levels, with RLX being more productive than CVD/CVDD.

The fact that RLX makes lumbar ALP activity go up suggests that it might help increase bone formation when PHT and SVP cause bone loss.

TRAP activity was much higher in the vertebral column of mice given 35 mg/kg of PHT or 300 mg/kg of SVP (p0.001). When used alone, neither RLX nor CVD/CVDD had much of an effect on lumbar TRAP activity. However, both were able to lower the increased levels of TRAP in the therapeutic and preventative groups.

As a way to prevent or treat bone loss, RLX Osteoclast-Mediated Bone Resorption

The amount of hydroxyproline (HxP) in the lumbar vertebrae of animals given PHT (35 mg/kg) or SVP (300 mg/kg) was much lower than in the control group (p0.001). HxP levels increased in the RLX, CVD, and CVDD intervention groups, but RLX did a better job raising HxP levels than either CVD or CVDD. On their own, these treatments did not change lumbar HxP levels very much.

RLX might help lessen the loss of mechanical bone strength caused by AEDs.

Compared to the control group, the U-Ca was significantly (p0.001) higher in the group that got PHT (35 mg/kg) and SVP (300 mg/kg). Both preventative and curative treatment with RLX led to a big drop in the amount of Ca^{2+} in the urine (p0.001). With CVD/CVDD, similar results were seen, but they were less strong (p0.05) than with RLX. Because AEDs cause less calcium to leave the body, RLX slows down bone turnover.

5.4 THE PURPOSE OF THIS STUDY IS TO EXAMINE RALOXIFENE'S INFLUENCE ON THE AED-INDUCING PROPERTIES OF ELECTROSHOCK-INDUCED CONVULSIONS

PHT 35 mg/kg, SVP 300 mg/kg, and LTM 200 mg/kg stopped all electroshock induced seizures, as shown by the loss of tonic hind limb extension (HLE). Responses caused by the combination of AEDs and RLX were the same as those caused by AEDs alone, and they did not change the tonic HLE seen during seizure activity in any significant way. Raloxifene did not make either of the AEDs less effective at stopping seizures. There was no statistically significant difference in the time it took to reach a tonic HLE or how long it lasted.

Since RLX does not affect how well AEDs stop seizures, it can't have pharmacodynamic communication with these drugs when used for a long time.

From above findings, we can figure out the following:

Raloxifene can also treat or stop bone loss and PHT and SVP.

5.5 DETERMINING WHETHER OESTROGEN AND TRANSFORMING GROWTH FACTOR BETA 3 (TGF3) MEDIATE THE EFFECTS OF AEDS AND RALOXIFENE ON BONE WAS THE FOCUS OF THIS STUDY

Research aimed at comprehending estrogen-mediated bone maintenance has focused on the cellular and molecular targets of estrogen action in bone. Previous research suggested that estrogen might affect the expression of regional elements crucial to maintaining skeletal homeostasis in order to carry out its biological effects in bone. We investigated the modulation of the TGFP by 17P-estradiol or a selective ER modulator (SERM), raloxifene, in zCzm and zCtro in order to discover target genes of estrogen regulation in bone. Our findings shed light on the processes by which raloxifene and estrogen affect bone.

When mice were given PHT (35 mg/kg) and SVP (300 mg/kg) over a long period, serum estradiol levels dropped by a lot (p0.001). Post-exposure prophylaxis (RLX and CVD) and medicinal (RLX and CVDD) treatments raised the low levels of oestrogen in the blood. When given on their own, RLX and CVD/CVDD did not significantly affect the amount of oestrogen in the blood.

Low levels of oestrogen may have worsened the bone loss caused by PHT and SVP.

Despite being treated with LTM (200 mg/kg p.o.) for four months, plasma estradiol levels did not change.LTM didn't change the levels of estradiol or the way the bones changed.

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Curriculum Vitae

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- Presently working as Assistant Professor in Raj Kumar Goel Institute of Technology (Pharmacy), Ghaziabad, Uttar Pradesh. The works performed includes Processing of classes, labs and coordinating, implementing and commissioning of various Project.
- I have completed Masters in Pharmacology from Banasthali Vidhyapith with Distinction.
- I have attended Faculty Development Programmes at many levels along with many International & national conferences and attained many prices.
- I have also attended FDP on Universal Human values.
- There are many publications of research articles as well as review articles under my name in Scopus Index and many other reputed journals along with many book chapters and book with authorized publishers.
- I have also experience on working on various software's such as Ex-Pharm and many other Microsoft tools.

I hereby declare the above details are true to the best of my knowledge and belief.

Your sincerely
Sagarika Kabra

List of Publications

Research Articles Published

- A Research Article Published in Latin American Journal of Pharmacy October 2023, 42(4) under heading "**The Portrayal of Drug Raloxifene by Using Histopathological Evaluation Methods in the deterrence of Post-Menopausal Osteoporosis through Biomarkers".**

- A Research Article Published in Biological Forum – An International Journal under UGC Care Indexed Journal List on heading "**The Effect of Raloxifene given in Post Menopausal Osteoporosis against the effects of Anti-Epileptics with the help of DEXA analysis and other Pathways"** 15(4):20-24(2023).

- I have also published a book chapter "Osteoporosis: The Role in Bone Health with its Therapeutic Potential" in book Women Health and Diseases A Challenge, Published by AB Publishers.

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The Effect of Raloxifene given in Post Menopausal Osteoporosis against the effects of Anti-Epileptics with the help of DEXA analysis and other Pathways

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ABSTRACT: The disease Epilepsy is known to be a common neurological disorder which is affecting majority of population in the whole world. The disease is caused due to many factors which includes abnormalities in brain, stroke, head injury, drug abuse, brain infection. Therefore, to treat such kind of disorders anti-epileptic drugs are used in major as well as minor quantity. By take excessive intake of anti-epileptic drugs it has shown to reduce bone density and bone mineral concentration in people who are taking these drugs on regular basis. As Anti-Epileptic Drugs have been known to be in association with negative impact on the bone health which leads to many kinds of bone disorders. Many kinds of biochemical abnormalities of bone metabolism are seen in patients taking these drugs. There were many kinds of challenges faced during the study as the Bone Mineral Density done using DEXA analysis was the measurement of soft tissue that is changes in femur and lumbar vertebrae. There was a contribution seen by the university in terms of apparatus/chemicals and other necessary requirements.
As if by seeing all the negative values it is found that the drug named Raloxifene has found to be a pre-eminent gem which has reduced the chances of bone density and other bone related disorders.

Keywords: Raloxifene, Anti-epileptic, DEXA, Sodium Valproate, Phenytoin.

INTRODUCTION

The disease termed as "Epilepsy" is known to be a most common neurologic disorder affecting majority of people in the whole world (Johannessen Landmark and Patsalos 2010). For treating such kind of disease there is need of first line treatment which is done by the administration of anti-epileptic drugs (AEDs) with that they are divided into three generations-first, second third generations. The most frequently used AEDs are Phenytoin (PHT), Pheno-barbital (PB), carbamazepine (CBZ), Valproic acid (VPA) & Clobazam (CLB). According to the International League Against Epilepsy it is known as the disease of the brain which is occurring due to majority of reasons such as at least two unprovoked seizures which are coming more that twenty-four hours apart; in which one is unprovoked seizure with the chances of more seizures over the next years.

Bone Structure and its Metabolism. Bone, a major contributing tissue of body that is perpetually remoulded throughout the life time of the individual (Holick and Krane 2001). There are specialized cells known as osteoblasts they help in initiating the formation of bone in which osteocytes detects bone mechanical stress & osteoclasts helps in bone resorption. The determination of bone density is done by the absolute balance in between the bone formation & bone resorption. The process of bone formation starts with the accumulation of organic matrix through osteoblasts which is accompanied by the process of mineralization. The organic matrix is composed of mainly type I collagen and other proteins.

There are a majority of biochemical markers that can be measured and they show the complete rate of bone remodelling.

Epilepsy: Bone Health in Women. Women are on major urge of epilepsy (Ohta *et al.*, 1992). It is found that the women with epilepsy are more prone and come with more problems in relation to bone health which results with low Bone Mineral Density as compared to males. The deficiency of estrogen which is either due to late in first menstrual cycle, primary / secondary amenorrhea as these are the major risk factors which leads to enhanced bone loss in women.

Many studies show that women lose around 35-50% of their complete bone mass in full period of their life (Riggs *et al.*, 1981). As before menopause this loss is very less and involves particular cancellous bone but during the 3-4 years of preceding menopause as well as

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The Portrayal of Drug Raloxifene by Using Histopathological Evaluation Methods in the deterrence of Post-Menopausal Osteoporosis through Biomarkers

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Abstract:
A disorder names "Osteoporosis" takes place due to variation's or disproportions in the activity of osteoblasts that is the bone forming cells. The aim of the study is to perform the histopathological analysis of drug Raloxifene using Bone biomarkers and bone regulators as they help to easily asses the osteoporosis in female mice through the evaluation of various biological parameters. There is use of Biomarkers for detecting the degradation of bone and then with the help of Bone Turnover markers it is easy to compare the effect of drug Raloxifene in treating the bone dysfunction and how this drug has shown its effects in people who are taking long term effect of Anti-Epileptic Drugs. The conclusion from the study states that Raloxifene helps to counteract the changes in bone and its loss on the basis of its evaluation parameters with the help of biomarkers it helps in the characterization which involves in early assessment of osteoporosis as this is only possible by collective assessment with DEXA and biomarkers. Therefore, Raloxifene is found to be selective estrogen receptor modulator having great results in females.

Keywords: Osteoporosis, Biomarkers, Histopathological Analysis, Raloxifene.

Introduction

The disease Osteoporosis is known to be caused due to the differences or imbalance in the process of bone remodeling which is known to be a continuous process in which the fully grown up bone tissue is removed by osteoclasts cells and the advanced or new bone tissue is again formed by new bone forming cells called osteoblasts as shown in Fig. 1. If there is uncontrolled or inadequate new bone formation during remodeling process this further leads to osteoporosis. Therefore, for maintaining the balance between both the kind of cells they should work in coordination with the help of many kinds of molecules Raisz *et al.* (2005).

The biomarkers of bone turnover are known to be worked in the previous years, in that the working of bone remodeling is mainly constituted by the bone resorption and the bone formation cells. The bone biomarkers are produced from the process of bone remodelling which includes bone formation biomarkers, bone resorption biomarkers & regulators of bone turnover. Therefore, to detect the process of bone metabolism this has been studies with the help of biomarkers of enzymes, proteins & by-products which are there at the time of bone remodelling process Shi X *et al.* (2014).

Various effects of epilepsy one Bone

Epilepsy increases the risk for fracture by a variety of mechanisms in addition to those attributed to the use of AEDs. The fracture rate in patients with epilepsy is 2–6 times higher than the rate observed in the general population Setoain J *et al.* (2001).

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