INTRODUCTION

Depression is a major cause of illness burden to societies worldwide with increasing effects on personal suffering, work disability, and use of healthcare resources ***(Wittchen et al., 2011)***. The current advances in basic and clinical research highlighted the potential role of new biological factors that may affect mood in combination with the more traditional neurochemical and neuroendocrine mechanisms ***(Annweiler et al., 2009)***. Many studies have given an increasing amount of attention to a possible role of vitamin D in cognitive function and mental health ***(Kalueff et al., 2007).***

Vitamin D is a unique neurosteroid hormone that may have an important role in the development of depression. Vitamin D receptors are present on neurons and glia in many areas of the brain including the cingulate cortex and hippocampus, which have been implicated in the pathophysiology of depression ***(Eyles et al., 2013).*** Vitamin D is also involved in numerous brain processes including neuroimmunomodulation, regulation of neurotrophic factors, neuroprotection, neuroplasticity and brain development making it biologically plausible that this vitamin might be associated with depression ***(Fernandes et al., 2009).***

Early deficiencies of vitamin D have been linked with neuropsychiatric disorders such as schizophrenia, and adult deficiencies have been associated with Parkinson's disease,

Alzheimer's disease, depression, and cognitive decline ***(Llewellyn et al., 2009).***

Epidemiological evidence concerning the association between vitamin D and depression is limited and non-conclusive: several studies identified associations between depression and low vitamin D levels, but there were also a number of studies with non-significant results ***(Parker & Brotchie, 2011)***. A recently published meta-analysis showed that lower vitamin D levels were significantly associated with greater risk of depression in noninstitutionalized individuals ***(Anglin et al., 2013)***. Proposed biological mechanisms for this association include that vitamin D has receptors that are distributed in brain areas involved in emotional processing and affective disorders ***(Eyles et al., 2013)***, regulates serotonin synthesis via transcriptional activation of the tryptophan hydroxylase 2 gene ***(Patrick & Ames, 2014)*** and impacts innate immunity and the production of proinflammatory cytokines that in turn influence mood by activating the stress response ***(Zhang et al., 2012).***

AIM OF THE WORK

- To measure serum vitamin D level in patients diagnosed as major depressive disorder according to DSM-IV criteria.
- To identify the possible impact of serum vitamin D level on severity of depression.

Chapter 1

VITAMIN D

Sources of vitamin D:

Vitamin D is fat-soluble steroids that are provided from natural synthesis in the skin and can be obtained from dietary sources, or pharmacologic supplementation ***(Spiro & Buttriss, 2014)***. The main source of vitamin D comes from the endogenous production while the diet is less effective source that is responsible for only 20% of the body needs ***(Leventis & Patel, 2008).***

Vitamin D may be consumed in the diet as either ergocalciferol (vitamin D2) from plant sources or cholecalciferol (vitamin D3) from animal sources. Relatively few foods are naturally rich in vitamin D. The main dietary sources of Vitamin D3 are dairy, egg, fish and meat. Vitamin D3 also comes from endogenous sources where it can be synthesized by skin on exposure to ultraviolet B irradiation ***(Mehrotra et al., 2014)***. Vitamin D2 cannot be synthesized endogenously, and comes from some plant foods such as edible mushroom grown in natural environment and artificially synthesized compounds ***(Urbain et al., 2011).***

Skin production of vitamin D is affected by skin condition, pigmentation and many environmental factors

resulting in limited access to sunlight, such as latitude, season, cloudiness or air pollution ***(Webb, 2006).***

Modern lifestyles often reduce the time in sunlight and decrease the opportunities to produce vitamin D endogenously in sufficient quantities. The concentration of vitamin D also decreases significantly during winter in non-equatorial locations in the absence of adequate supplementation or suitable fortification ***(Vieth, 2004).***

Vitamin D metabolism:

The cutaneous precursor of vitamin D; 7-dehydrocholesterol, undergoes photochemical cleavage of the carbon bond between carbons 9 and 10 of the steroid ring through sunlight exposure, giving rise to previtamin-D3 which is converted into vitamin D3 (cholecalciferol) within 48 hours. Vitamins D2 and D3 from dietary sources are transported to the liver on chylomicrons, while vitamin D3 from cutaneous production is carried through plasma bound mainly to a vitamin D-binding protein and a small fraction is bound to albumin. Vitamin D is biologically inactive, it is activated in a two-stage hydroxylation process as shown in figure (1). It is hydroxylated in the liver on position C-25 by 25-hydroxylase and converted into 25-hydroxyvitamin D [25(OH) D]; calcidiol ***(Bringhurst et al., 2008).***

Subsequent hydroxylation occurs in the cells of the convoluted proximal tubule of the kidneys by the 1α-hydroxylase enzyme resulting in formation of 1,25-dihydroxyvitamin D [1,25(OH) 2D]; calcitriol. 1,25(OH)D is finally metabolized in the kidneys, where it is transformed initially into 24,25(OH)2D3, then into 1,24,25(OH)2D3 [1,24,25-dihydorxyvitamin D] and finally into calcitroic acid, which is excreted through urine ***(Campbell, 2014).***

25(OH) D, calcidiol, is the dominant metabolite of vitamin D in the circulation and 1,25(OH) 2D, calcitriol, is the biological active form of vitamin D. The hormonal activity of calcitriol is exerted through binding to the nuclear vitamin D receptor (VDR) ***(Bauer et al., 2013).*** 1,25(OH) 2D has relatively short half-life (~4 hours), while 25(OH)D has a long half-life (2-3 weeks), so vitamin D status is best monitored by 25(OH)D ***(Mousa et al., 2016).***

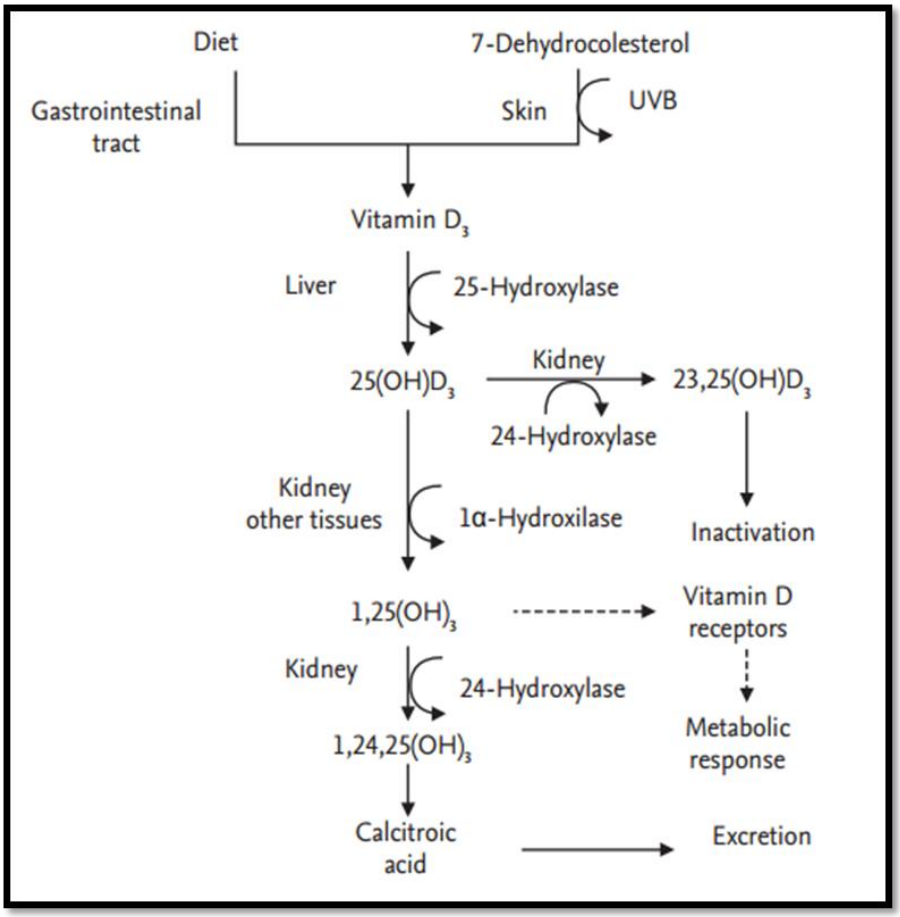

Figure (1): Schematic representation of vitamin D metabolism – *adopted from Korean, 2016.*

UVB, ultraviolet B; 25 (OH)D3, 25-hydroxyvitamin D.

Daily Requirements of vitamin D:

Vitamin D intake of 200-600 IU per day is recommended to prevent deficiency and the related health consequences. The intake of vitamin D has to be increased to 800 – 1000 IU per day in individuals without sunlight exposure. During winter months and for those living at high latitudes, dietary intake is

essential to maintain healthy 25(OH)D levels. While during sunny months in most parts of the world, vitamin D sufficiency can be achieved by minimal sun exposure. Vitamin D levels resulting from daily exposure of 50% of the skin without sunscreen for 12 minutes during mid-day hours at mid latitudes are equivalent to those produced by dietary intake of 3,000 IU per day ***(Holick, 2008).***

Biological activity of vitamin D:

Vitamin D has diverse biological functions through the binding of the biologically active metabolite; 1,25(OH)2D to nuclear vitamin D receptor (VDR) in target tissues to regulate gene transcription. 1,25(OH)2D also binds to VDRs on cell membranes to mediate a variety of non-genomic responses (***Norman, 2008***). VDRs control more than 200 genes and are expressed by a variety of cells, including the **epithelium of the small bowel** and **renal tubules**, **osteoblasts**, **osteoclasts**, **hematopoietic cells**, **lymphocytes**, **epidermal cells**, **pancreatic cells**, **myocytes**, and **neurons** ***(Szodoray et al., 2008).***

Vitamin D is involved in many physiological functions in addition to its classic role in bone metabolism:

1. **Mineral homeostasis:**
   - It increases the intestinal absorption of calcium.
   - It participates in calcium mobilization from the bones, in the presence of parathyroid hormone (PTH).

- It increases the renal reabsorption of calcium from the distal tubule ***(Bringhurst et al., 2008).***

2. Regulation of bone formation:

- It inhibits the synthesis of type 1 collagen.
- It induces the synthesis of osteocalcine.
- It promotes the differentiation of monocytes-macrophages precursors into osteoclasts.
- It facilitates maturation of osteoclast precursors into osteoclasts which mobilize calcium storage in the bones to maintain calcium homeostasis through stimulation of the production of Receptor activator of nuclear factor kappa-B ligand (RANK-L) (***Bringhurst et al., 2008***).

3. Immunomodulation:

- It modulates T-cell proliferation ***(Gombart, 2009).***
- It activates the genes encoding the antimicrobial peptides with natural features of antibiotics ***(Wang et al., 2004).***
- It is a repressor for interleukin and reduces the risk of some autoimmune diseases, such as rheumatoid arthritis and diabetes mellitus (type 1) ***(Anagnostis et al., 2013).***

4. Brain processes:

VDR and vitamin D activating enzyme, 1- alpha-hydroxylase, are widely distributed in human brain, particularly

in the hypothalamus and the limbic system. Accordingly, the brain locally activates vitamin D (***Bertone, 2009***). Vitamin D is involved in numerous brain processes including:

- Brain development, neuroimmunomodulation, regulation of neurotrophic factors and neuroplasticity ***(Fernandes et al., 2009).***
- Regulation of behavior and emotions ***(Kalueff et al., 2004).***
- Neuroprotection through regulation of calcium concentrations intra- and extracellularly in neurons, consequently reducing toxicity caused by excess calcium ***(Kalueff et al., 2004).***
- It may be directly involved in the autocrine or paracrine regulation of the brain ***(Eyles et al., 2013).***

Vitamin D status:

Serum 25(OH)D level is the best indicator of Vitamin D status reflecting both dietary intake and sunlight exposure. It is used because:

- It has longer half-life than that of 1,25(OH)2D.
- Its concentration is 1,000 times higher than that of 1,25(OH)2D.
- Its concentration remains unaffected while the concentrations of 1,25(OH)2D may remain within normal limits or might even be elevated in some cases due to increased production of 1,25(OH) 2D by the kidney due to

the compensatory increase of PTH secretion associated with vitamin D deficiency ***(Bandeira et al., 2006).***

Enzyme-linked immunosorbent assay (ELISA) was used previously to measure serum 25(OH)D level. Recently, Liquid chromatography–mass spectrometry (LC-MS/MS) became the gold standard of measuring vitamin D level due to:

- Its ability to differentiate and quantify accurately the two subgroups of 25(OH)D (25(OH)D2 and 25(OH)D3).
- High specificity, that allows differentiation of the target molecule from other metabolic intermediates, and avoiding interference as seen in immunological methods.
- High sensitivity, that allows detection at ng/mL level ***(Yang, 2012).***

The ideal serum level of vitamin D does not exist, but the levels of vitamin D should remain with a range that does not induce an increase in PTH levels. Normal levels vary according to the commercial assay used, from 25-37.5 nmol/L (10-15 ng/mL) to 137.5-162.5 nmol/L (55-65 ng/mL) ***(Leventis & Patel, 2008).***

Adequate vitamin D status (>30 nmol/L) significantly promotes calcium absorption and reduces rickets risks, which was deemed as good vitamin D nutritional status. Some researchers defined serum 25-OH vitamin D lower than 50

nmol/L as vitamin D deficiency, 50-75 nmol/L as inadequate, and higher than 75 nmol/L as adequate ***(Yu & Han, 2015).***

Disorders associated with vitamin D:

Inadequate vitamin D status is associated with multiple health risks. Several risk factors may predispose to vitamin D deficiency, including low exposure to sunlight, skin hyperpigmentation, low dietary intake, smoking, pollution, aging, sedentarism, intestinal malabsorption, kidney disease, liver disease, obesity and deficiency secondary to medications and genetic factors ***(Bidgoli & Azarshab, 2014).***

Approximately one billion people have either vitamin D insufficiency or deficiency ***(Khadilkar & Khadilkar, 2013).*** Prolonged vitamin D deficiency is detrimental to the skeleton, resulting in rickets in children and osteomalacia in adults ***(Thacher & Clarke, 2011).*** Vitamin D insufficiency may lead to secondary hyperparathyroidism, bone loss, muscle weakness and falls and fragility fractures in older people ***(NOS, 2013).***

It is also associated with non-skeletal disorders including hypertension, heart disease, diabetes mellitus, age-related cognitive decline, Parkinson's disease, multiple sclerosis, arthritis and cancer. However, whether low 25(OH)D is the cause or result of ill health is not yet clear ***(Autier et al., 2014).***

Vitamin D toxicity is a rare condition. Excessive intake of vitamin D, mainly through high doses of vitamin D

supplements has been shown to have toxic effects, while excessive sunlight exposure does not cause vitamin D toxicity. These toxic effects include hypercalcaemia and in certain cases renal failure and cardiac arrest. Signs of vitamin D intoxication include anorexia, vomiting, headache, calcification of soft tissues and formation of urinary calcium stones ***(NIH, 2011).***

Tolerable upper intake level that does not pose risks for public health is determined to be 25 μg/day for infants and children aged 10 years or younger and 50 μg/day from 11 years and older, while in adults the risk is increased in some parts of the population with a daily intake above 100 μg/day ***(Holick, 2007).***

Chapter 2

MAJOR DEPRESSIVE DISORDER

Depression is a chronic or recurrent condition impairing all aspects of human function. It is a common illness affecting more than 300 million people worldwide. The burden of depression is on the rise globally. It is associated with significant disability, mortality and healthcare costs. At its worst, depression can lead to suicide. 800 000 people die every year due to suicide. Although there are known effective treatments for depression, fewer than half of those affected in the world (in many countries, fewer than 10%) receive such treatments. Barriers to effective care include lack of resources, lack of trained health-care providers, and social stigma associated with mental disorders. Another barrier to effective care is inaccurate assessment. In countries of all income levels, people who are depressed are often not correctly diagnosed, and others who do not have the disorder are too often misdiagnosed and prescribed antidepressants ***(WHO, 2017).***

Prevalence of major depression:

Major depressive disorder (MDD) is common debilitating psychiatric disorder. It occurs in all cultures and affects all age groups. The mean age of onset is generally in the 30s, and childhood and late adult onsets are common ***(Kessler et al., 2003).***

The lifetime prevalence for major depression is reported to be as high as 14-17% and the one-year prevalence is 4-8%. It occurs in women twice as frequently as men, and the lifetime prevalence rates of MDD among women are 10-25%, and for men 5-12%. The prevalence of depressive episodes was reported to be 9.1%. The major risk factors for these episodes are age, marital status, number of diagnosed chronic diseases and alcohol consumption. Moreover, major depression is associated with threefold increased risk of premature mortality as compared to the general population ***(Fekadu et al., 2016).***

The prevalence of depression in children is relatively low (<1% in most studies), and then increases considerably all the way through adolescence with a one-year prevalence of 4–5% in mid to late adolescence. Depression is major risk factor for suicide observed in adolescents; it is one of the leading causes of death in this age group. Depression also leads to serious social and educational impairments and associated with an increased rate of smoking, substance abuse and obesity ***(Thapar et al., 2012).***

Clinical picture of major depression:

MDD is characterized by depressed mood or markedly diminished pleasure or interest in all activities for a consecutive period of 2 weeks, and associated with the presence of four or more of the following symptoms: