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Research Report

The combination of ultraviolet-B and vitamin K2 exposure effect on fibroblast-like cell number n Wistar rats

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ABSTRACT

Background: Vitamin D deficiency n children s one of the problems most often discussed and received attention from around the world today, especially during the Corona virus disease-19 (Covid-19) pandemic. Vitamin D3 ncreases the production of vitamin K2 protein and activates a protein nvolved n bone metabolism. Vitamin K2 ncreases bone formation by stimulating osteoblast differentiation, regulating mineralization of the extracellular matrix, regulating bone marker gene expression, and nhibiting osteoclastogenesis. **Purpose:** To analyzed the effect of sun exposure, Ultraviolet-B (UV-B) and vitamin K2 supplementation on fibroblast-like cell as bone formation marker n Wistar rats (Rattus novergicus). **Methods:** Twenty-four samples divided nto 4 groups namely control group, UV-B group, vitamin K2 group, combination of UV-B and vitamin K2 group. After 21 days, extraction of lower ncisors was done to examine fibroblast-like cell number after treatment. After being decalcified, specimens underwent histological evaluation using Haemotoxylin and Eosin staining to observe the fibroblast-like cell number. **Result:** Data analysis of fibroblast like cells number expression using one way analysis of variance (ANOVA) test showed a significant difference between sample groups ($p<0.05$). **Conclusion:** The combination of UV-B exposure and vitamin K2 administration group ncreased of fibroblast-like cells n Wistar rats (*R. novergicus*).

Keywords: fibroblast-like cells; UV-B; vitamin K2; COVID-19; medicine; dentistry

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INTRODUCTION

The South East Asian Nutrition Survey (SEA-nuts) data showed that children aged 2-4 years who experience vitamin D deficiency reached 42.8% n rural areas and 34.9 percent n urban areas. n the Southampton survey, a study found nvolving children as young as 4 years old, observed that they had a 1.4% higher bone mass density (BMD) for every additional 10 minutes of outdoor activity per day. n children aged 9-10 years, an additional 10 minutes of outdoor activity was associated with 1–2% higher bone mineral density.[1]

Vitamin D deficiency in children is one of the problems that is most often discussed and received attention from around the world today, especially with the corona virus disease-19 (COVID-19) pandemic. This is related to the existence of a health protocol where children are prohibited from doing activities outside the home, both in public places and schools. Various studies abroad both in sub-tropical and tropical countries show the prevalence of vitamin D deficiency in children is quite high.[2] In 2020, Nelwan et al conducted a study showing exposure to UV-B light and vitamin K2 supplements play a role in bone formation.[3] Nonetheless, there are limited report on vitamin D status in children or other groups. Severe vitamin D deficiency shows clinical signs such as rickets in children and low bone density. The increasing prevalence of vitamin D deficiency or insufficiency is partly due to the low intake of vitamin D sources, low fat food intake, increased use of sunscreen, and less exposure to sunlight.[4]

Vitamin K plays a role in the regulation of calcium metabolism in tissues, cell growth and proliferation, oxidative stress, inflammatory reactions, blood clotting and hemostasis.[5] Vitamin K2 prevents bone resorption through its anticatabolic activity, which reduces osteoclast differentiation and inhibits osteoblast apoptosis. Bone metabolism depends on the interaction between vitamins D3 and K2. Combining vitamins D and K will have a beneficial effect on calcium homeostasis which will improve

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bone quality.[6] Vitamin D3 increases the production of vitamin K2 protein and activates proteins involved in bone metabolism.[7]

The synthesis of vitamin D3 in the skin involves two stages, namely the photochemical conversion of 7-dehydrocholesterol to pre-vitamin D3, followed by isomerization of vitamin D3. Factors that influence changes in pre-vitamin D in the skin include skin pigmentation and intensity of exposure to ultraviolet light. In Indonesia, there is still very limited information on vitamin D status in both children and adults. Furthermore, outdoor physical activity is known to have a positive effect on bone development during childhood. the outdoor physical activity increases bone mass. In addition, previous study found that outdoor physical activity where there is an interaction with vitamin D (sun) in adequate doses during childhood will affect the process of bone mass improvement.[8]

International guidelines from the World Health Organization (WHO) recommend one hour of outdoor activity per day or at least three days a week in childhood to adolescence for strong bones. However, a number of studies indicate that children spend less time outdoors. The time that should be used for outdoor physical activity is replaced by an increase in the time children spend watching television or playing computer games which affects bone health.[9] Children who like outdoor physical activities have been shown to improve their brain abilities and academic achievement. This study involved school children aged 6-18 years. In the survey, researchers analyzed 21 reports containing four themes, namely fitness and health; intellectual ability; involvement; motivation and well-being. As a result, researchers concluded that physical activity and outdoor fitness are good for children's brain development and function.[10]

Most natural foods contain very low amounts of vitamin D3 and do not improve vitamin D3 status or meet recommendations for dietary vitamin D3 intake. Sun exposure on the skin is the best way for the synthesis of vitamin D from pre-vitamin D found under the skin. On the skin exposed to ultraviolet light will convert vitamin D into essential nutrients. UVB light with a wavelength of 290-315 nm, which comes from the sun will be absorbed by the skin and will then convert 7-dehydrocholesterol in the skin into previtamin D3, which will then spontaneously be converted into vitamin D3 and so on will undergo metabolism in the liver to become 25(2)(OH)D and in the kidneys to 1,25(OH)2D3.[11] The main role of vitamin D, which has been most widely known so far, is maintaining bone mineralization, known as the calciotropic effect, regulating calcium and phosphate metabolism in the small intestine, osteoblasts, kidneys and parathyroid glands.[12] Previous study that have been carried out include the role of ultraviolet B (UV-B) sunlight on vitamin D status and blood pressure in women of childbearing age.[13] However, there is limited study that discuss sun exposure and vitamin K2 supplements on bone formation. Based on this background, this study was conducted to analyze sun exposure on bone formation in the teeth of Wistar rats (Rattus novergicus) that received vitamin K2 supplements.

MATERIALS AND METHODS

The study design was a laboratory experimental research. n this study sampling technique used random sampling techniques. This study used twenty-four Wistar rats (R. novergicus), with the nclusion criteria were 120-200 grams, 2-3 months old, generally n good condition, adapted for 1 week. 45 mcg of Vitamin K2 (Doctor's Best, US) supplements that were given to the sample once a day. Besides administering the vitamin K, the samples were expose to the 290-315 nm UV-B exposure (Phillips) for

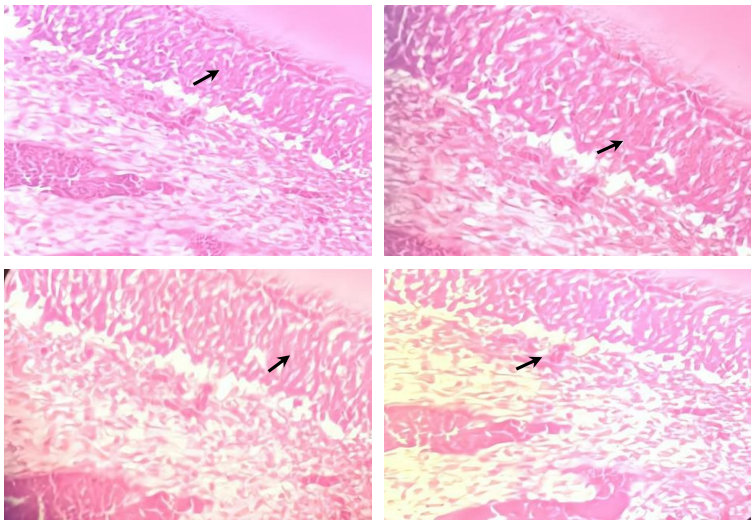

Figure 1. Histopathology anatomy examination showed fibroblast-like cell pulp tissue of Wistar rats treated with 400x imagnification (black arrow indicates fibroblast-like cell) with small, oval, and dark cell nucleus morphology with oval and long cytoplasm. A. no UVB exposure, B. no UV-B exposure with vitamin K supplementation, C. UV-B exposure group, D. UV-B exposure with vitamin K supplementation group.

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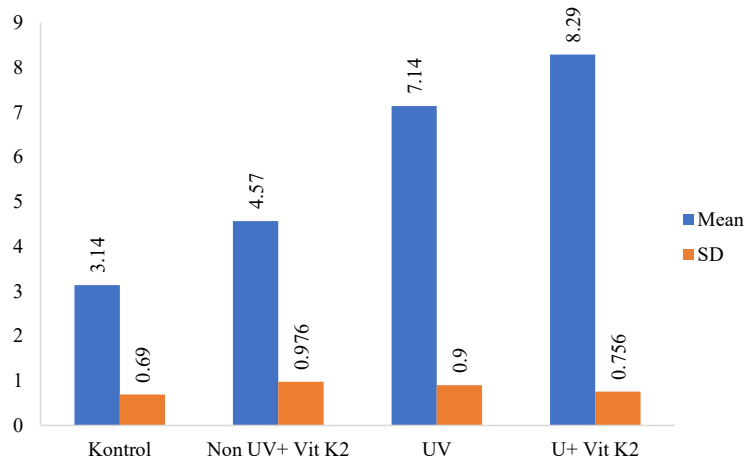

Figure 2. Mean of the fibroblast-like cells in each groups.

Table 1. One-way ANOVA difference test

	Sum of Squares	Mean Square	Sig.
Between Groups	115.857	38.619	0.000*
Within Groups	16.857	0.702	
Total	132.714		

*Information: significant at p<0.05

25 minutes, three times a week. Twenty-four sample wereidivided nto four experimental groups: no UVB exposure (Kontrol group); UV-B exposure group; UV-B exposure with vitamin K supplementation group; no UV-B exposure with vitamin K supplementation. After treatment, all sampleiwere sacrified then the tooth extraction was done from the lower left ncisor. Decalcification was performed on the tooth for 30 day. HE staining was done to calculate the fibroblast like cell by means of light microscope. The morphology of fibroblast-like s small, oval, dark cell nucleus morphology with an oval and long cytoplasm. The data were recapitulated and statistically analyze by means of analysis of variance (p<0.05).

RESULTS

In this study the marker of bone formation used was the number formation of fibroblast-like cells where the formation of this variable was the mmature formation before the osteoblast. Fibroblast-like cells were found n each group (Figure 1). The UV-B exposure with vitamin K administration has the greatest number of fibroblast-like cells (Figure 2). There was significant different of fibroblast-like cells number between group (P<0.05) (Table 1).

DISCUSSION

The highest average of fibroblast-like cells number was found in the UV-B and vitamin K2 exposure group. This might happens due to the bone metabolism depends on the interaction between vitamins D3 and K2. Vitamin D3 increases the production of the protein vitamin K2, while vitamin K2 activates proteins involved in bone metabolism. Vitamin K2 acts as a co-factor in the carboxylation of *glutamic acid* (Glu) to Gla and the metabolically active form of osteocalcin, which can bind and store calcium in the extracellular matrix.[7] In addition, it was found that vitamin K2 promotes the differentiation and proliferation of osteoblasts. Fibroblast-like cell is a formation that occurs before the process of maturation into osteoblasts. Osteoblasts are bone-forming cells that originate from progenitor cells and are found on the surface of bones. These cells are responsible for the formation and process of bone mineralization. Osteoblasts originate from pluripotent mesenchymal stem cells and these cells can also develop into chondrocytes, adipocytes, myoblasts, and fibroblasts. Osteoblasts synthesize collagen and *glycosaminoglycans* (GAGs) from the bone matrix and play a role in the process of bone mineralization.[14]

Under certain conditions such as wound healing, UV radiation causes the proliferation of melanocytes which are important for wound closure. Melanocytes are known to secrete a wide variety of *keratinocyte growth factor* (KGF) and cytokines such as interleukin (IL)-1, IL-6, IL-8, and *transforming growth factor alpha* (TGF-α) following UV stimulation, all of which induce mitogenic activity in epidermal keratinocytes. Wound healing is a very dynamic, complex physiological process, which aims to build the integrity of the damaged tissue. Healing involves phases of homeostasis, inflammation, granulation, fibrogenesis, re-epithelialization, neovascularization, and maturation. Controlled exposure to UV light is beneficial for wound healing.[15]

Vitamin D that comes from consumption or is produced endogenously from exposure to UV light on the skin is hydroxylated first in the liver and then again in the proximal kidney tubule to be converted into its metabolically active form. Vitamin D deficiency contributes to the development of hypocalcemia and hypophosphatemia. PTH, which is secreted by the parathyroid glands, plays a role in the hormonal regulation of calcium (Ca^{2+}). Increased parathyroid hormone (PTH) secretion as a result of hypocalcemia works to return serum Ca^{2+} to the normal range by increasing bone formation, via osteoblasts-osteoclasts to release Ca^{2+} into the bloodstream.[16] Thus, hypophosphatemia due to vitamin D deficiency results in low concentrations of the mineral ions Ca^{2+} and Pi preventing proper mineralization of the organic bone matrix and loss of 1,25(OH)2D signaling to mineralized cells that contributes to the lack of mineralization of the resulting bone and teeth.[17]

Vitamin K2 can improve bone quality and reduce the risk of fractures as shown by many studies with population groups over the age of 50. In addition, in children who are born with vitamin K2 deficiency and are not repaired, bone formation will be not optimal. This is evidenced by mutations in vitamin K-dependent enzymes, which result in defects that affect bone and cartilage development. In the bone marrow mesenchymal stem, vitamin K2 administration supports osteogenic differentiation.[18]

In this study, a dose of vitamin K2 of 45 mcg was used which was adjusted to the body weight of rats, which was 120-200 gram. Vitamin K2 helps bone formation by stimulating osteoblast differentiation, increasing levels of bone anabolic markers and regulating extracellular matrix mineralization via -glutamyl carboxylase. In addition, vitamin K2 also plays a role in preventing bone resorption by reducing osteoclast differentiation and inhibiting osteoblast apoptosis.[19]

The physiology of bone metabolism relies on synergistic interactions between vitamins D3 and K2. Bone metabolism depends on the interaction between vitamins D3 and K2. Vitamin D increases the production of vitamin K protein, while vitamin K activates proteins involved in bone metabolism. Vitamin K acts as a cofactor in the carboxylation of glutamic acid (Glu) to Gla and the metabolically active form OC, which can bind and store calcium in the extracellular matrix. Based on previous research, if only vitamin D3 will not affect the proliferation of osteoblasts.[20]

In this study, the variable used as a marker is fibroblast-like cell where this cell is an immature form of osteoblast. These fibroblast-like cells are in direct contact with blood vessels which are a source of nutrition for teeth and bones.[21] Fibroblast-like cells have been widely used in tissue engineering studies of blood vessels and skin because of their ability to secrete extracellular matrix proteins and aid in wound healing. Fibroblast-like cells are found in many dental tissues, including pulp, milk teeth, periodontal ligament, papillae, dental follicles, gingival tissue.[22] There is ample evidence supporting the osteoprotective effect of vitamin K2 on bone metabolism. Vitamin K2, especially MK-4, promotes bone formation by stimulating osteoblast differentiation, regulating the mineralization of the extracellular matrix, regulating the expression of bone marker genes, and inhibiting osteoclastogenesis.[23] Based on this study result, t can be concluded that the combination of UV-B exposure and vitamin K2 administration group ncreased of fibroblast-like cells n Wistar rats (*R. novergicus*).

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