Association of Serum Vitamin D Levels on Response Therapy in Patient Lung Adenocarcinoma Advanced with Targeted Therapy

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Abstract

Objective: This study aimed to analysis the associated of serum vitamin D levels and response therapy in advanced lung adenocarcinoma patient after used TKIs. **Methods:** This study was an observational analytic study. The subjects where patient with advanced lung adenocarcinoma who received tyrosine kinase inhibitors (TKIs) for 3 months at Dr. Soetomo general hospital Surabaya from July to March 2021 who met the inclusion and exclusion criterias. The independent variable in this study are the serum levels of vitamin D and response therapy objective (RECIST criteria) as the dependent variable. **Results:** The results of stastitical analysis showed that there was no significant associated of serum vitamin D levels and response therapy ($p>0.05$). Patient insufficiency with partial response had a greater number than patient sufficiency with partial response. The mean of vitamin D levels in patient with progressive disease was the highest. **Conclusion:** Although the serum levels of vitamin D in lung cancer was lower but in this study showed that there was no significant associated of serum vitamin D levels and response therapy in patient advanced lung adenocarcinoma with tyrosine kinase inhibitors.

Keywords: *Lung adenocarcinoma, vitamin D, response therapy, tyrosine kinase inhibitors*

Introduction

Lung cancer is one of the most frequently diagnosed cancers worldwide, with 12.9% of all new case findings found and 19.4% of all cancer deaths or around 1.2 million deaths globally each year[1, 2]. Currently, therapeutic modalities in the field of oncology are very advanced, but the prognosis for lung cancer remains very poor, with a 5-year survival rate in the United States of 16% and less than 10% in the UK [2, 3]. Most of the lung cancers found are at an advanced stage, namely stage IIIB and stage IV[3]. The working potential of vitamin D in the prevention and treatment of cancer has now been developed through various studies. Optimal vitamin D status plays an important role in the prevention of lung cancer because in addition to the kidneys, vitamin D is converted into a form of active metabolites in other organs, one of which is the lungs[1, 4].

Epidemiological studies suggest that vitamin D deficiency is associated with an increased incidence of cancer and poorer therapeutic outcomes, although many studies have not demonstrated this association. Vitamin D deficiency is associated with a poor prognosis in NSCLC lung cancer patients receiving platinum-based

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chemotherapy. The role of vitamin D itself is associated with the inhibition of cancer cell development because it has anti-tumor and immunomodulatory effects. Vitamin D plays a role in important functions of cells, such as: cell proliferation, apoptosis, differentiation, metastasis, and angiogenesis[1, 5]. Previous epidemiological studies have shown that vitamin D is associated with a reduced risk of colorectal cancer and breast cancer. Findings from in vitro studies suggest that vitamin D can decrease cell proliferation and can induce apoptosis of small cell lung carcinoma (SCLC)[5, 6]. Two other analogues of vitamin D, namely PRI-2202 (24R calcipotriol) and PRI-2205 (5,6 trans calcipotriol) when combined with cisplatin are more effective in inhibiting the growth and spread of lung cancer[7]. Values of higher baseline vitamin D levels have a better effect on vitamin D supplementation[8].

There are many research findings regarding the benefits of vitamin D, but there is still very limited research on the benefits of vitamin D in the development of lung cancer therapy and there are studies on the role of vitamin D in inhibiting the EGFR pathway to prevent cell proliferation, induce apoptosis, inhibit differentiation and metastasis and angiogenesis of cancer cells. lung which prompted investigators to further investigate the relationship between the value of vitamin D levels in serum and response to therapy in patients with advanced pulmonary adenocarcinoma with targeted therapy.

Method

Participants included patients with advanced adenocarcinoma with EGFR mutations who received targeted therapy. Participant inclusion criteria included a diagnosis of non-small cell lung carcinoma (NSCLC) at IIIB, IIIC, and IV statium with EGFR mutations based on histopathological or cytological results, had never been targeted for therapy, had initial thoracic CT scan data at a distance of approximately 1 month before giving targeted therapy, AST and ALT <3× normal values, and serum creatinine values <1.5× normal values. The exclusion criteria for participants included patients who had received chemotherapy/radiotherapy before, or received combination with other therapies such as resection surgery, radiotherapy, immunotherapy, and patients who did not continue targeted therapy until prior to CT scan evaluation after 3 months of targeted therapy.

This type of research is a prospective longitudinal observational analytic study conducted in the period July 2020 to March 2021. The number of participants was 30 participants using the calculation of the correlation coefficient estimation formula and consecutive sampling technique. Participants were assessed for participant characteristics, vitamin D levels, and post therapy responses to the provision of targeted therapy for 3 months. We have done ethical approval in the Ethical committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (2000/KEPK/V/2020).

Participants' vitamin D levels were measured using CMIA (Chemiluminescent Microparticle Immunoassay) with the Architect i2000 SR immunoanalyzer, which is measured in ng/mL units. Vitamin D levels are divided into four categories as follows: deficiency with a level <10 ng/mL, insufficiency in the range 10-<30 ng/mL, deficiency with a level of 30-100 ng/mL, and toxicity with a level >100 ng/mL.

Response therapy was evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 which was categorized into 4, namely progressive disease (PD), stable disease (SD), partial response (PR), and complete response (CR)[9, 10]. The results of the research are displayed in the form of figures or tables. The statistical analysis used was the Chi Square test where $p < 0.05$. Statistical analysis used IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA).

Result

Participant Characteristics

Most of the participants were male as many as 17 participants (54.8%) and most of them were aged in the range 46-55 years as many as 18 participants (58.1%). Most of the participants worked indoors or indoors as many as 24 participants (77.4%) and most of the participants were exposed to cigarette smoke (61.3%).

Based on this data, 24 participants (77.4%) chose to be exposed to sunlight at 07.00-09.00. During the duration of receiving sun exposure, 11 participants (35.5%) were ≤15 minutes. Most of the participants were exposed to sunlight for 5-7 days in 1 week (58.1%). Most of the participants had EGFR mutation exon 19 delese (67.7%) and received Gefitinib therapy (61.3%; table 1).

Participant Serum Vitamin D Levels

As many as 25 participants (80.6%) had levels of vitamin D in the insufficiency category and the rest in the category of sufficiency.

Therapeutic Response in Advanced-Stage Pulmonary Adenocarcinoma Patients

Participants had a therapeutic response as follows PR in 18 patients (58.1%), SD as many as 10 patients (32.3%), and PD as many as 3 patients (9.7%). Differences in therapy (EGFR-TKIs) obtained in study patients also resulted in different responses, as in Table 2.

Association between Vitamin D Levels and Participant Therapy Response

Table 2 shows that there were patients with insufficient vitamin D levels but showed PR in 14 patients (77.8%), on the other hand there were also patients with PD who showed vitamin D deficiency in 2 patients (66.7%). There was no significant relationship between vitamin D levels in serum and treatment response in patients with advanced pulmonary adenocarcinoma ($p >$ 1.000).

Tabel 1. Patient Characteristics

Category	Total
Gender	
Male	17 (54.8)
Female	14 (45.2)
Age	
36-45 years old	2 (6.5)
46-55 years old	18 (58.1)
56-65 years old	8 (25.8)
66-75 years old	2 (6.5)
76-85 years old	1 (3.2)
Type of work	
Indoor	24 (77.4)
Outdoor	7 (22.6)
Cigarette Exposure	
Yes	19 (*61.3*)
No	12 (38.7)
Sun Exposure Time	
07.00-09.00 am	24 (77.4)
09.00-11.00 am	6 (19.4)
None	1 (3.2)

Cont... **Tabel 1. Patient Characteristics**

Duration of sun exposure	
≤15 minutes	11 (35.5)
16-30 minutes	10 (32.3)
≥31 minutes	9 (29.0)
None	1 (3.2)
Frequency of sun exposure per week	
1-2 days	6 (19.4)
3-4 days	6 (19.4)
5-7 days	18 (58.1)
None	1 (3.2)
EGFR mutations	
18 G719A, 21 L858R	1 (3.2)
18 G719D, 19 Del, 21 861Q	1 (3.2)
18 G719X, 20 S768I	1 (3.2)
19 Del	21 (67.7)
19 Del, 21 L816Q	1 (3.2)
21 L858R	4 (12.9)
21 L861Q	1 (3.2)
21 L858R, 21 L861Q	1 (3.2)
EGFR TKI	
Afatinib	11 (35.5)
Gefitinib	19 (61.3)
Erlotinib	1 (3.2)

Table 2. Therapeutic EGFR-TKIs with a therapeutic response

	PD	**SD**	**PR**	**p**
EGFR-TKI				0.189
Afatinib	0 (0.0)	2 (18.2)	9 (81.8)	
Gefitinib	3 (15.8)	7 (36.8)	9 (47.4)	
Erlotinib	0 (0.0)	1 (100.0)	0 (0.0)	
Vitamin D				1.000
Insufisiensi	14 (77.8)	10 (100.0)	1 (33.3)	
Susfisiensi	4 (22.2)	0 (0.0)	2 (66.7)	

Discussion

The half-life of calcidiol 25(OH)D-formed vitamin D is 2–3 weeks, whereas the plasma calcitriol 1,25(OH) D2 is <4 hours[11]. Many factors affect vitamin D such as intake factors in the form of sun exposure, food or supplement intake and factors that affect the metabolism of vitamin D itself such as decreased synthesis in the skin (use of sunscreen, skin pigment color, aging process), decreased bioaviability (obesity, increased body fat), increased catabolism (use of anticonvulsants, glucocorticoids), decreased synthesis of 25(OH)D (liver failure), increased excretion of 25(OH)D through urine (nephrotic syndrome), Other diseases: Hyperthyroidism that causes decreased concentrations 25(OH)D due to increased 25(OH)D2 metabolism[12].

Vitamin D levels associated with clinical response to neoadjuvant chemotherapy in postmenopausal women with loccaly advanced breast cancer found that 43.3% of patients had vitamin D levels before chemotherapy and 56.7% of patients had vitamin D deficiency[13, 14]. There are no studies that explain the effect of TKIs on vitamin D metabolism. Chemotherapy of cisplatin in endometrial cancer patients affects calcitriol levels but not calcidiol. This is due to the suppression of 1α-OHase activity not due to global protein synthesis by anti-cancer agents but due to renal damage that occurs[15].

Cancer cells develop resistance to vitamin D by modulating the expression of VDR, CYP27B1, CYP24A1 in the vitamin D metabolic pathway. VDR is widely expressed in several tissue cells, one of which is lung, but this expression decreases during tumor differentiation and development in cancer cells. Cancer cells bind or ligate on E-boxes (E-boxes are one of the DNA response elements present on the binding-site protein which functions to regulate gene expression in tissue) which are present in the proximal promoter region of the VDR gene to recruit co-repressors there is inhibition of transcription of the VDR gene[4, 16]. Another method of modulation of cancer cells against VDR is by binding VDR directly or indirectly (mediated transcriptional program) through the mutated tumor suppressor gene p53 so that cancer cells can avoid apoptosis or natural cell death. The P53 suppressor gene can increase VDR expression, but in cancer cells, the P53 tumor suppression gene is reduced or in some cancer cells, the P53 gene has a mutation[16]. Cancer cells can also modulate VDR by suppressing VDR transcription through the expression of the K-RAS gene mutation in cancer cells. Cancer cells inhibit CYP27B1 expression so that the active formation of 1,25(OH)2D3 is reduced to prevent the anti-proliferative effect caused by 1,25(OH)2D3. Mawer et al found that only 1 cell out of 16 small cell lung cancer cells was capable of synthesizing 1,25(OH)2D3. This shows that lung cancer cells inhibit CYP27B1 expression, so that the active formation of 1,25(OH)2D3 is reduced and this is necessary for cancer cells to prevent anti-proliferative effects. However, an increase in CYP27B1 expression can occur in cancer because it suppresses and avoids the immune system by increasing the secretion of active vitamin D as well as a feedback from the body's response to activating 1,25(OH)2D3[16, 17]. Cancer cells increase the expression of CYP24A1, CYP24A1 expression 8-50x higher than normal lung tissue and the worse the degree of differentiation of a tumor, the higher the expression of the CYP24A1 enzyme. There are several mechanisms of CYP24A1 induction by cancer cells. Increased CYP24A1 micro-RNA (mRNA) in lung tumors compared to normal lung tissue[4, 17]. The miR-17 to miR-92 clusters regulate CYP24A1 expression in lung cancer cells[16].

In the LUX-Lung 7 study comparing "head-to-head" between Afatinib and Gefitinib, Afatinib showed a slight extension of Progression Free Survival/PFS (time from initial diagnosis of disease to progression or disease severity), 11 months vs 10.9 months compared to Gefitinib. Median time-to-treatment failure (time to treatment failure) was also longer for Afatinib (13.7 months vs 11.5 months, $p = 0.0073$) with a better objective response rate (70% vs 56%, $p = 0.0083$)[18]. The superior effect of Afatinib is known due to the EGFR mutation that occurs mostly in exon 19[19]. In addition, Afatinib irreversibly and equipotently inhibits

the intrinsic activity of all receptors of the ErbB group (EGFR, HER2 and ErbB4) and indirectly inhibits ErbB3 by preventing binding (ligand-dependent) phosphorylation of ErbB3[20].

Adenocarcinoma patients with EGFR mutations will initially respond to EGFR-TKIs therapy, but will subsequently develop resistance to TKIs over a period of 9-14 months[21, 22]. There was little difference in the overall survival of NSCLC patients given platinum-based chemotherapy in the group with serum vitamin D values <20 ng/mL compared to the group with vitamin D values ≥20 ng/mL (19.5 vs 22.6 months). The PFS in the group with serum vitamin D values <20 ng/mL compared to the group with vitamin D values ≥20 ng/mL were almost the same, namely with a median value of 9.4 vs 9.8 months[23].

Most patients with vitamin D insufficiency could respond to therapy in the form of a partial response because most patients had exon deletion 19 mutations and the mean PFS EGFR mutation exon 19 was greater than exon 21 (9.05 vs 6.76 months)[24]. Patients with EGFR mutation exon 19 gave a significantly better response after administering TKIs than exon 21[5]. There are 3 hypotheses that underlie the better effectiveness of exon 19, namely first because exon 19 has a greater affinity for the EGF receptor than exon 21 so that the bonds formed between TKIs and exon 19 are stronger and signal barriers for cancer cell growth will last longer. The second hypothesis is that the secondary mutation T790M occurs more frequently with exon 21, especially L858R, which is a point mutation with the largest proportion in exon 21. The third hypothesis is that mutations in exon 18 (G719S) occur more frequently with exon 21 (L858R)[5, 25].

Conclusion

There was no significant relationship between vitamin D levels in serum and response to therapy in patients with advanced pulmonary adenocarcinoma with targeted therapy for TKIs for 3 months. The value of vitamin D levels in patients with advanced pulmonary adenocarcinoma is mostly insufficient. The response to therapy in patients with advanced lung adenocarcinoma was obtained mostly in the form of a PR of 58.1%, 32.3% of other therapeutic responses to SD and 9.7% of PD.

Funding: None.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: We have conducted an ethical approval base on Declaration of Helsinki at Ethical Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (2000/KEPK/V/2020).

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