

RESEARCH ARTICLE

Serumal and Salivary 25(OH)D and 1,25(OH)D Levels of Head and Neck Cancer Patients

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Abstract

BACKGROUND: Saliva has been suggested as a substitute of serum for the detection of 25 Dihydroxyvitamin D (25(OH)D) in healthy people. However, investigation of salivary 1,25(OH)D has not been clearly reported. Vitamin plays important roles in inhibiting cancer progression. Current study was conducted to investigate serumal and salivary 25(OH)D and 1,25(OH)D levels of healthy and head and neck cancer (HNC) subjects.

METHODS: Research were conducted at Haji Adam Malik Hospital, Medan, Indonesia. Forty HNC and 40 healthy subjects were recruited and selected based on inclusion and exclusion criteria. Medical records were documented, followed by anthropometric evaluation and serum and saliva collection. Laboratory investigation for 25(OH)D and 1,25(OH) was performed using Enzyme-linked immunosorbent assay (ELISA) methods.

RESULTS: Significant serumal ($p=0.002$) and salivary ($p=0.016$) 25(OH)D mean level differences of HNC and normal groups were obtained. More serumal or salivary 25(OH)D deficient subjects were found in control group than those in HNC group. Meanwhile, serumal and salivary 1,25(OH)D mean levels of HNC group were not significantly different with the ones of control group. There were significant correlations of serumal-salivary 25(OH)D as well as serumal-salivary 1,25(OH)D levels in normal group.

CONCLUSION: Serumal and salivary 25(OH)D and 1,25(OH)D levels of HNC group were relatively normal. Salivary 25(OH)D and 1,25(OH)D could be suggested as substitutes for serumal ones.

KEYWORDS: vitamin D, 25(OH)D, 1,25(OH)D, head and neck cancer

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Introduction

Most head and neck cancer (HNC) patients are deficient in vitamin D which might be associated with incidence of tumors.(1) Vitamin D could inhibit tumor cell proliferation, induction of apoptosis and cell cycle arrest, as well as increase immune system and sensitivity to chemotherapeutic

agents.(2-4) In addition, vitamin D supplementation was suggested to suppress tumor development and cellular activity.(1)

HNC is the sixth most common cancer worldwide and is one of the ten most common cancers in men.(5) There were 249,330 cancer survivors in 2019, 3% of all cancer patients. The number of survivors is expected to increase to 315,750 in January 2030.(6) HNC represent the third

most prevalent cancer in Indonesia (9.14%), after the breast (16%) and cervical cancers (10.86%).(7)

Vitamin D could increase T cell activation by increasing the expression of CD4, CD8, and CD69.(8,9) Vitamin D is mainly activated by the binding of 1 α ,25 Dihydroxyvitamin D (1,25(OH)D) (active form) or 25(OH) D (less active form) to the Vitamin D Receptor (VDR), including nuclear receptor members of regulatory genes, which have anti-inflammatory and anti-proliferation activities.(10-12)

Routine examinations of vitamin D levels are often carried out to check the serumal 25(OH)D level, although the active form of vitamin D is 1,25(OH)D. This active form is produced predominantly by the kidneys. Damage to blood vessel cells in the kidneys will cause a deficiency in serumal 1,25(OH)D level.(13) In patients with impaired renal function, this active form can be disrupted, which could be found in patients with HNC.(13,14)

Serumal and salivary 25(OH)D levels were moderately correlated in healthy people (14,15), which was also shown by various metabolic parameters (16). Meanwhile, serumal and salivary 1,25(OH)D levels were not correlated in healthy people. However, salivary 25(OH) D and 1,25(OH)D levels of HNC patients have not been disclosed clearly. Therefore, current study was conducted to compare the levels of serumal-salivary 25(OH)D and 1,25(OH)D of HNC patients and healthy people. This study also investigated the possibility of using saliva as a non-invasive sample for detection of 25(OH)D and 1,25(OH)D in HNC patients.

Methods

Participants

Current study was conducted in Haji Adam Malik Hospital, Medan, Indonesia, from January 2020 to July 2021. Sampling data during the period of the COVID-19 pandemic was carried out following strict health protocols, which was approved by the Universitas Sumatera Utara Ethical Committee (No. 63/KEP/USU/2020) and registered at Clinical Trials.gov (No. NCT04655664; date of document: November 18, 2020). All subjects have signed informed consent to participate in this study.

Forty HNC and 40 healthy subjects were recruited. Study subjects with the age of 18–60 years old were included. Subjects who took vitamin D supplementation, having chronic pain or metabolic disorders, such as impaired liver or kidney function were excluded.

Anthropometric Examination

Body mass index (BMI) was categorized based on Asia Pacific values: <18.5 kg/m² as underweight, 18.5–22.9 kg/m² as normoweight, 23–24.9 kg/m² as overweight/at risk, 25–29.9 kg/m² as obese I, and >30 kg/m² as obese II.(17)

Serum and Saliva Collection

For determination of serumal 25(OH)D and 1,25(OH), 2 mL of blood was drawn into a tube without coagulant, left for 15 minutes, centrifuged at 3000 rpm for 10 minutes and stored at –20°C for the test.(18)

For determination of salivary 25(OH)D and 1,25(OH), 2 mL saliva was collected from 90-minutes fasting subjects between 09.00-11.00 AM without stimulation. Saliva collection procedures were followed.(18) Saliva was centrifuged at 2500 rpm for 10 minutes, and stored at –20°C.

25(OH)D and 1,25(OH) Enzyme-linked Immunosorbent Assay (ELISA) and Analysis

Both serumal and salivary samples were examined for 25(OH)D using Human 25-Dihydroxy Vitamin D ELISA Kit (Cat# E1981Hu, Bioassay Technology Laboratory, Shanghai China). Basically, the 25(OH)D antibody-precoated wells were added with samples. And then biotinylated human 25-OH-D antibody was added and bound to 25(OH)D in the sample. Then Streptavidin-HRP was added and bound to the Biotinylated 25(OH)D antibody. Substrate solution was then added. The reaction was terminated by addition of acidic stop solution and the absorbance was measured at 450 nm with Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Fischer Scientific, Porto Salvo, Portugal).

For 1,25(OH)D examination, Human 1,25-hydroxyvitamin D ELISA Kit (Cat# E1919Hu, Bioassay Technology Laboratory) was used. In principle all conducted procedures were similar to the ELISA procedure for detection of 25(OH)D, by using 1,25(OH)D antibody-precoated wells and biotinylated 1,25(OH)D antibody.

Serumal and salivary 25(OH)D levels were defined as deficient: <12 ng/mL, insufficient: 12-20 ng/mL, and optimal: >20 ng/mL. Serumal and salivary 1,25(OH)D levels were defined as deficient: ≤48 pmol/L and normal: >48 pmol/L.(19,20)

Statistical Analysis

Descriptive statistics for continuous variables were summarized as means±standard deviations (SD), and the categorical variables were summarized by proportions. Testing was carried out using Mann–Whitney test numerical

data, Chi-Square test for categorical data. Fisher's exact test was used for the data unmet Chi-Square criteria. The Spearman correlation test was used for the data not normally distributed.

Results

Due to the COVID-19 pandemic, there were decreasing number of visiting head and neck cancer (HNC) patients and large number of infected health workers. Over a period of six months, 40 patients were selected based on the inclusion criteria. Equal number of healthy subjects was recruited as well. Subject demographic characteristics were shown in

Table 1. Mostly, both HNC and control subjects were in the 46-60 years old group, male, entrepreneur, and senior high school graduates. with normal body mass index. Most HNC subjects were Bataknesse while control subjects were Malay. Most HNC subjects were having normal body mass index (BMI) while control subjects were obese. HNC cases were mostly found in nasopharynx than lymph node with keratinizing SCC histopathologic type (Table 2). Most cases were found in IIIA stadium with lymph disorder. Mostly subjects underwent chemotherapy and had co-morbid diseases.

Significant serual and salivary 25(OH)D mean level differences of HNC and normal groups were obtained ($p < 0.05$, Mann-Whitney test). Both serual and salivary

Table 1. Demographic characteristics of all subjects (n=80).

Parameters	HNC Group	Control Group	p- value
Age (years) [mean±SD]	46.4±12.1	41.18±11.14	0.645 ^a
Age group [(n (%))]			
18–25 years	1 (2.5)	4 (10.0)	1.980 ^b
26–35 years	6 (15.0)	6 (15.0)	
36–45 years	6 (15.0)	11(27.5)	
46–60 years	27 (67.5)	19 (47.5)	
Genders [(n (%))]			
Male	30 (75.0)	22 (55.0)	0.006 ^{b,*}
Female	10 (25.0)	18 (45.0)	
Ethnic [(n (%))]			
Bataknesse	20 (50.0)	18 (45.0)	0.001 ^{b,*}
Javanese	14 (35.0)	0 (0.0)	
Malay	3 (7.5)	22 (55.0)	
Nias	2 (5.0)	0 (0.0)	
Acehnese	1 (2.5)	0 (0.0)	
Occupation [(n (%))]			
Entrepreneur	30 (75.0)	18 (45.0)	0.008 ^{b,*}
Housewife	4 (10.0)	13 (32.5)	
State civil apparatus	2 (5.0)	6 (15.0)	
Student	1 (2.5)	3 (7.5)	
No job	3(7.5)	0(0.0)	
Education [(n (%))]			
Master Program	0 (0)	2 (5.0)	3.290 ^b
Undergraduate	4 (10.0)	7 (17.5)	
Senior High School	27 (67.5)	22 (55.0)	
Junior High School	8 (20.0)	6 (15.0)	
Primary School	1 (2.5)	3 (7.5)	
BMI (kg/m ²) [mean±SD]	20.76±3.36	27.13±13.76	0.001 ^{a,*}
BMI category [(n (%))]			
Underweight	7 (17.5)	3 (7.5)	0.002 ^{b,*}
Normal	26 (65.0)	13 (32.5)	
Overweight	3 (7.5)	8 (20.0)	
Obese	4 (10.0)	16 (40.0)	

* $p < 0.005$, ^aIndependent T test, ^bChi-square.

25(OH)D mean levels of HNC group were significantly higher than those of control group (Table 3). More serumal or salivary 25(OH)D deficient subjects were found in control group than those in HNC group. Meanwhile, serumal and salivary 1,25(OH)D mean levels of HNC group were not significantly different with the ones of control group.

Based on Spearman's analysis showed that there was no correlation of the serumal-salivary 25(OH)D as well as serumal-salivary 1,25(OH)D levels in HNC group (Table 4). In contrary, significant correlations of serumal-salivary 25(OH)D as well as serumal-salivary 1,25(OH)D levels in normal group were found.

Discussion

In current study, there were more men than women (5,21), who were reported to have two-fold greater risk of developing HNC than women due to tobacco consumption, alcohol consumption, oropharyngeal cavity hygiene, and human papillomavirus (HR-HPV) infection (3,5,9). Most nasopharyngeal keratinizing squamous cell carcinoma was found in current study, while in other studies, different types of primary tumors and stages of cancer were focused.(5,10,22)

In current study, the serumal and salivary 25(OH)D levels of HNC group were significantly higher than those of control group. These findings were different from the reported study showing vitamin D levels of HNC patients were lower than those of controls. This might be related to the control group whether having the risk of metabolic disorders associated with low vitamin D levels. In addition, although the levels were higher in HNC group, these

Table 2. Characteristics of HNC subjects (n=40).

Variables	n (%)
HNC cases	
Nasopharynx	39 (97.5)
Lymph node	1 (2.5)
Histopathologic type	
Undifferentiated SCC	2 (5)
Keratinizing SCC	21 (52.5)
Non-Keratinizing SCC	17 (42.5)
Stadium of HNC	
II	2 (5)
IIIA	21 (52.5)
IIIB	14 (35)
IV	3 (7.5)
Lymph disorder	
No	3 (7.5)
Yes	37 (92.5)
Chemotherapy	
No	4 (10)
Yes	36 (90)
Co-Morbid diseases	
No	4 (10)
Yes	36 (90)

serumal and salivary 25(OH)D levels of HNC group were mostly categorized as insufficient. Meanwhile serumal and salivary 1,25(OH)D levels of HNC and control subjects were categorized as normal. This active form of vitamin D is important in regulating only related to bone density, but also with incidence decrease of HNC tumor growth; function increase of the immune system, decrease of cell proliferation, induction of apoptosis, and cell cycle arrest, and increase of sensitivity to chemotherapeutic agents.

Table 3. Serumal and salivary 25(OH)D and 1,25(OH)D levels.

Parameters	Serumal			Salivary		
	HNC Group	Control Group	p- value	HNC Group	Control Group	p- value
25(OH)D level (ng/mL) [mean±SD]	15.71±9.07	12.0±10.45	0.002 ^{c,*}	21.63±10.96	15.57±5.51	0.016 ^{c,*}
25(OH)D category [(n (%))]						
<12 ng/mL (Deficiency)	13 (32.5)	28 (70.0)	0.002 ^{b,*}	4 (10.0)	11 (27.5)	0.025 ^{b,*}
12–20 ng/mL (Insufficiency)	20 (50.0)	7 (17.5)		18 (45.0)	21 (52.5)	
>20 ng/mL (Optimal)	7 (17.5)	5 (12.5)		18 (45.0)	8 (20.0)	
1,25(OH)D level (pmol/L) [mean±SD]	162.04±75.54	235.83±180.60	0.260 ^c	214.08±92.72	198.46±53.92	0.954 ^c
1,25(OH)D category [(n (%))]						
≤48 pmol/L (Deficiency)	2 (5.0)	0 (0)	0.152 ^d	0 (0)	0 (0)	Not analyzed
>48 pmol/L (Normal)	38 (95.0)	40 (100)		40 (100)	40 (100)	

* $p < 0.005$, ^bChi-square test, ^cMann–Whitney test, ^dFisher Exact Test.

Table 4. Spearman's Correlation between serumal or salivary 25(OH)D or 1,25(OH)D levels.

Variable	HNC Group		Control Group	
	Serumal 25(OH)D level	Serumal 1,25(OH)D level	Serumal 25 (OH) D level	Serumal 1,25 (OH) D level
Salivary 25(OH)D level	$r=-0.090, p=0.579$	-	$r=0.402, p=0.010^*$	-
Salivary 1,25(OH)D level	-	$r=0.069, p=0.672$	-	$r=0.383, p=0.015^*$

* $p<0.005$.

(3,9) Therefore, although the serumal and salivary 25(OH)D levels were insufficient, but the serumal and salivary 1,25(OH)D levels were normal.

Serumal and salivary 25(OH)D levels were significantly correlated in control group. Serumal and salivary 1,25(OH)D levels were significantly correlated in control group as well. Current results are in accordance with previous report, which showed a correlation between serumal and salivary 25(OH)D levels.

There were some barriers and limitations in current study. Due to the COVID-19 pandemic, all processes starting from the subject recruitment and screening, sample collection and laboratory investigation took longer than the schedule. Although collection of saliva was not invasive, however optimal saliva collection could not be performed for the subjects with hyposalivation. More clinical laboratory assessments should be performed to evaluate kidney and liver functions.

Conclusion

Serumal and salivary 25(OH)D levels were significantly higher in the HNC group than in the control group. Although serumal and salivary 25(OH)D levels of HNC group were insufficient, but the serumal and salivary 1,25(OH)D levels were normal. Serumal-salivary 25(OH)D as well as 1,25(OH)D levels were correlated in normal group. Taken together, in current study, the serumal and salivary 25(OH)D and 1,25(OH)D levels of HNC group were relatively normal. Salivary 25(OH)D and 1,25(OH)D could be suggested as substitutes for serumal ones.

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Authors Contribution

DKS, ZL and RAG were involved in research conception, DKS and FF performed the data acquisition/collection. LMS and ERD calculated the experimental data and performed the analysis. LMS did the manuscript preparation. LIL and FS aided in interpreting the results. FF and FS drafted the manuscript and designed the figures. All authors took parts in giving critical revision of the manuscript. All authors already gave final approval of the version to be published; and agreed to be held accountable for all aspects of the work.

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