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## Salivary Protein Profiling of Head and Neck Cancer Patients at West Nusa Tenggara Province General Hospital: A preliminary study based on single dimension SDS-PAGE analysis

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# Salivary Protein Profiling of Head and Neck Cancer Patients at West Nusa Tenggara Province General Hospital: A preliminary study based on single dimension SDS-PAGE analysis

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**Abstract.** The initial aim of this study was to investigate the protein profiles found in the saliva of head and neck cancer (HNC) patients at the Regional General Hospital of West Nusa Tenggara Province, Indonesia. Saliva samples were collected from 21 patients with head and neck cancer (confirmed from the results of anatomical and pathology examination). Mainly the types of HNC are sinonasal and nasopharyngeal cancers. As the control, saliva samples from 10 healthy individuals were used. Each subject thoroughly rinsed their mouth with a commercial mineral water and around 2-3 mL of un-stimulated saliva was collected in a 10 mL disposable plastic container with screw cap and immediately placed on ice. Sodium azide and protease inhibitors were added into the collected saliva and then transported to the laboratory within 1 hour of sample collection and centrifuged. The supernatant was taken; the protein concentration was measured, and then lyophilized. Protein profiles were analyzed using 1D-SDS-PAGE at 12.5% running gel. The data obtained were analyzed descriptively. In this preliminary study protein bands with a molecular weight range of 35-63 kDa were observed in the saliva of HNC patients, which were not found in the saliva of healthy individuals. Whether the expression of the specific protein bands can be used as a non-invasive biomarker in patients with head and neck cancer; needs to be investigated further.

## 1. Introduction

Head and neck cancer (HNC) is the most common cancer found in developing countries, especially in Southeast Asia, and is the sixth most common cancer in the world [1]. In Indonesia, HNC cancer is in the fourth position, and nasopharyngeal and sinonasal cancers are common among all head and neck cancers, with an incidence of 6.2 per 100,000 population, so there are an estimated 15,500 new cases in one year [2].

In West Nusa Tenggara (WNT) Province with a population of around 4 million people, it is estimated that around 400 new cases will be found annually [3]. In 2015, it was found at least 48 cases of WHO III type or undifferentiated HNC type in all districts or cities in WNT Province, with the most cases coming from West Lombok Regency.



Sinonasal tumor cases are also quite common in WNT, in 2015 there were 8 new cases with 97 hospital visits [4]. It was understood that high morbidity and mortality in cancer patients with HNC were mainly due to delayed or slow diagnosis [5, 6]. In this regard, especially in an effort to detect HNC, with non-invasive biological material, it was mentioned that studies on the proteomics of saliva patients with HNC play an important role in establishing biomarkers for the early detection of HNC [7].

A preliminary study on the profiling of protein saliva of patients suffered from head and neck cancer at West Nusa Tenggara Province General Hospital has therefore been conducted based on single dimension SDS-PAGE analysis.

## 2. Materials and Methods

### 2.1. Collection of saliva.

Ethical approval of this study was obtained from the ethics committee of the Faculty of Medical School, University of Mataram No. 46/UN18.F7/ETIK/2019.

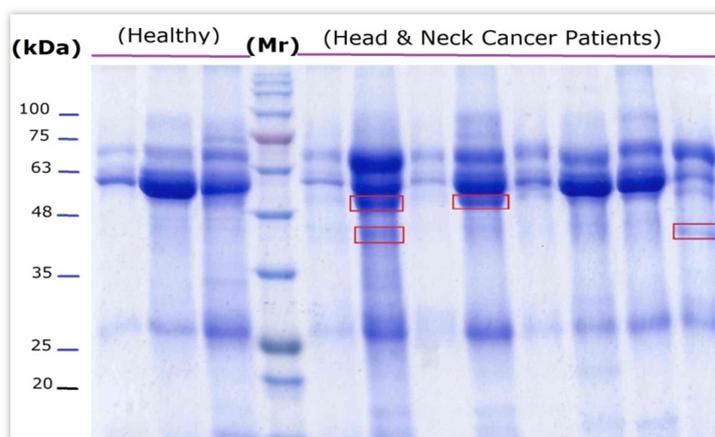
Saliva samples were collected from patients diagnosed with nasopharyngeal and sinonasal cancer at the General Hospital of West Nusa Tenggara Province, Mataram, Indonesia. The criteria for nasopharyngeal and sinonasal cancer are histopathological examination with the existence of malignant cells. In addition no treatment has been taken to the subjects in the form of chemotherapy and/or radiotherapy. Saliva of healthy subjects that were those who did not fall into those categories was also collected as controls. The sampling method used was purposive random sampling, with as many as 21 cancer patients and 10 healthy subjects were used as the controls. Before collecting the saliva, each subject thoroughly rinsed their mouth with a commercial mineral water, after that about 2-3 mL of un-stimulated saliva was collected in a 10 mL disposable plastic container with screw cap and immediately placed on ice. Sodium azide and protease inhibitors were added into the collected saliva and then transported to the laboratory within 1 hour of sample collection and centrifuged. The supernatant was taken; the protein concentration was measured, and then lyophilized for further analysis.

### 2.2. Protein electrophoresis.

One dimension electrophoresis gel (1D.SDS-PAGE) was performed according to [8], in a discontinuous gel system. The final polyacrylamide gels concentrations were 12.5% (w/v) for the running gel and 4.5% (w/v) for the stacking gel. Further details of the methods used in gel preparation and electrophoresis were described previously [9]. Briefly one hundred micrograms of each fraction (40  $\mu$ L) of saliva patient or healthy subject were mixed with 10  $\mu$ L of 5 x SDS-PAGE loading buffer (0.5 M Tris, pH 6.8, 10% SDS, 38% glycerol, and 0.1% bromophenol blue). Subsequently the samples were immediately heated to 95°C for 5 min, allowed to cool to room temperature. Each sample was loaded onto the SDS-PAGE separation gel and the electrophoresis was then carried out at 45 mV until the bromophenol blue front reached the bottom of the gel. Following this, the saliva proteins were stained with commassie brilliant blue (CBB) Stain One (Nacalai Tesque) according to the manufacturer's procedure. The protein bands derived from saliva of cancer patients were then compared to the bands of those of healthy subjects' saliva. The results were analyzed descriptively.

## 3. Results and Discussions

Representation of the result is presented in figure 1. It was observed that there were three clusters of molecular weight in the range of about 14-18 kDa, 35-40 kDa, and around 50-60 kDa (molecular weight marker PageRuler; Thermo Scientific, Waltham, MA, USA). From these three clusters, only one clusters was observed in the saliva of healthy subjects i.e. proteins with molecular weights of around 50-60 kDa.



**Figure 1.** Representation of salivary protein profiles of healthy subjects vs. HNC-suffered subjects analyzed in 1D-SDSPAGE (12.5%). Red squares indicate typical bands on HNC subjects, which do not expressed in the saliva of healthy subjects.

Interestingly, in this study, proteins in the range of 14-18 kDa and 35-40 kDa were not expressed, or if present, they were weakly expressed in the saliva of healthy subjects. Meanwhile, protein bands in the range of 40-55 kDa were clearly observed in the saliva originating from patients with HNC (Figure 1, red squares).

Previous studies reported that some proteins possibly existed, both in the HNC and healthy subjects, but their amount possibly decrease or increase significantly [10] such as S100 calcium binding protein (MW~12kDa), transferrin (~80kDa), or cofilin-1 (~18kDa) were found to be significantly increased in the saliva from squamous cell carcinoma of the head and neck (SCCHN) samples compared to the control group, whereas transthyretin or TTR (MW~55kDa) was significantly decreased. The main responsibility of TTR is for the transport of the thyroxine and retinol-retinol complex (RBP complex) to various parts of the body and brain, reviewed in [11]. Although in this preliminary study a specific biomarker (s) has not yet been elucidated, profiling protein in the saliva of HNC patients at the Regional General Hospital of WNT Province known to be comparable to the molecular weight range for HNSCC saliva (37-68 kDa) that have been reported previously [12]. Whether the proteins in our current study are related to the proteins reported previously, need to be elucidated further.

#### 4. Conclusions

Expression of protein bands in patient with head neck cancer at the Regional General Hospital of WNT Province have been revealed through 1D-SDSPAGE successfully. Whether the expression of the protein bands can be used as a non-invasive biomarker in patients with head and neck cancer; needs to be investigated further.

#### 5. Acknowledgements

This study was supported by a grant from the General Directorate of Higher Education, Ministry of Research and Technology Republic of Indonesia (National Competitive Basic Research Grant No. 7/E/KPT/2019/No. 1834/UN18.L1/PP/2019). The authors are grateful to the Director and the staff of the General Hospital of WNT Province for the contribution in samples collection, as well as to Mr. Suparman and Mr. Khalid for their technical assistance.

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## 2019 International Conference on Advances in Biological Science and Technology

### Preface

The conference proceedings contain the selected papers presented at the 2019 International Conference on Advances in Biological Science and Technology (ICABST2019). The conference is organized by Asia-Pacific Association of Natural Science and Engineering and Maejo University (Thailand), which has been successfully held in Bangkok, Thailand during December 26-28, 2019.

As an annual gathering, it provides an extensive platform for scientists, researchers and scholars to present their research results and newest findings in all fields of Biological Science and Technology, discuss the practical challenges encountered recommend better solutions for biotechnology. ICABST2019 includes keynote speeches, invited speeches, oral presentations and poster presentations. It provided unique opportunities for scientists to introduce novel ideas and studies in the following topics:

- ▶ Molecular Biology
- ▶ Biomechanics and Biomechanical Engineering
- ▶ Biomedicine, Biopharmaceuticals and Pharmacology
- ▶ Agricultural Science, Animal & Plant Science
- ▶ Bioinformatics & Biocomputing
- ▶ Application of biotechnology
- ▶ Other Related Topics

The conference lasted for 2 days including Invited Session, five Oral Sessions and two Poster Sessions. During the invited session, Prof. Gil Navon (Tel-Aviv University) with H-index of 52 & over 10000 times citations, gave an invited talk entitled "Molecular Imaging of Breast Cancer by Chemical Exchange Saturation Transfer MRI of Glucose Analogs". Then Prof. Hadi Shafiee (Harvard Medical School) gave a talk about "Mobile Health Diagnostics with Applications in Medicine". At last, Prof. Taisun Kim (CEO of Biometrix Technology Inc.; Professor of Hallym University) also gave a wonderful speeches with the title of "6 HCV Genotyping 9G test™ for highly accurate screening and genotyping of six HCV genotypes including HCV 1a, 1b, 2, 3, 4 and 6 (6a, 6f, 6i and 6n)". After invited session finished, Dr. Pompun Vivithanaporn (Mahidol University), as the session chair, made a detailed conclusion of all invited speeches; accompanying a heated discussion.

This conference proceeding presents a selection from papers submitted to the conference from universities, research institutes and industries. All of the papers were subjected to peer-review by conference committee members and international reviewers. The papers selected depended on their quality and their relevancy to the conference. The volume tends to present to the readers the recent advances in the field of resources and environmental research and various related areas.

The Organizing Committee expresses deep gratitude to all organizers and participants of the conference for their active participation, interest and discussion which is a significant contribution to the development of biotechnology.

Jason Guowei Sun  
Asia-Pacific Association of Natural Science and Engineering, China  
March 5, 2020





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