

Study on Extraction and application of human saliva protein

Xiao Hao, Yansong Zhang, Wei Sun, Huaijie Zhu*

The Second Affiliated Hospital of Zhengzhou University, 2# Jingba Road, Zhengzhou, Henan 450014, China.

*Corresponding Author: Huaijie Zhu, MD, Email; jacksun689@gmail.com, Tel. 86-150-3711-5732

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Abstract: Objective: Collection and extraction of human saliva proteins; **Material and Methods:** Random from health examination population, different gender, and different age saliva supply 60 people as the research object using saliva supply. According to the age divided into 30-40, 41-50, and 51-60 three age groups, that group were composed with 1/2 male and 1/2 female. Collect saliva 200 UL. Used Jacksun Easy Biotech Inc. Saliva Protein Extract Kit, catalog number is JZ-013, following the kit protocol exactly. To be in 30ul RIPA solution /each person respectively. Results: Saliva protein extracted from 200 UL can be obtained saliva protein of 30 UL, 4-4.27ug/ul. 30-40 years old group of saliva before breakfast to obtain the highest concentration protein extraction. Conclusion; 1. 200ul saliva can extract 30ul, 4-4.27ug/ul protein; 2. Saliva protein content was highest in 30 minutes before breakfast; 3. Saliva collection and protein extraction are easy and convenient, which is the most convenient source of protein for human diseases.

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Keywords: Saliva, Saliva Protein Extract, Biomarker, Protein Marker. Diagnosis, Human Disease.

1. Introduction:

With the development of modern molecular biology, more and more attention has been paid to the study of biological protein markers in the research, diagnosis and treatment of human diseases^{1, 2}. It is one of the most urgent problems that how to obtain the protein from the human body and use it in the research and application of biological protein markers³. This study reports the extraction and application of human saliva proteins.

2. Material and Methods:

2.1. Object: Random from health examination population, different gender, and different age saliva supply 60 people as the research object using saliva supply. According to the age divided into 30-40, 41-50, and 51-60 three age groups.

2.2. Saliva collection:

2.2.1. Human whole unstimulated saliva was collected by spitting method without swallowing, Saliva was collected 30 minutes before breakfast, lunch and dinner;

2.2.2. The supply in the collection of saliva requirements before dinner, have breakfast and lunch, after brushing, mouthwash, and / or use a toothpick, as far as possible to clean teeth and oral meal retained food residue, and remind saliva supply not eat any food in the saliva was collected before;

2.2.3. Before the collection, the saliva supplier sees the prepared breakfast, and the dinner, and reminds the saliva provider to collect the saliva, so as to be able to eat;

2.2.4. 200-250 UL / person saliva was collected with sterile containers, placed at room temperature for 3-5 minutes, with a pipette, 200 UL no bubble of saliva to move to 1.5 ml sterile centrifugal tube, to be used to extract saliva protein immediately, or to keep the 200ul at 4 C refrigerator, and to be used for saliva protein extract within a week.

2.3. Extract of the Saliva:

Used Jacksun Easy Biotech Inc. Saliva Protein Extract Kit, its catalog number is JZ-013, <http://www.jacksunbio.com>. Following the kit protocol exactly. To obtain the Protein in 30ul RIPA /each person respectively. To obtain the protein concentration to be ug/ul, see below;

2.4. Measuring the protein Concentration; Saliva protein estimation was done based on the Biuret method⁴. Protein forms a colored complex with cupric ions in alkaline medium. Based on this principle, salivary protein estimation was done by mixing the ready saliva protein 1ul with the reagent (45 g of Rochelle salt and 15 g of copper sulfate in 400 mL of 0.2 N sodium hydroxide. Five grams of potassium iodide was added to make up to 1 L with 0.2 N sodium hydroxide) 999 ul, and measuring the colored product using a photoelectric colorimeter at a wavelength of

546 nm. Standard solution of 6 g of bovine albumin dissolved in 100 mL of normal saline containing 0.1 g/dL sodium azide was used.

3. Results:

3.1. Determination of saliva protein content of 30 UL extracted from saliva of 200ul:

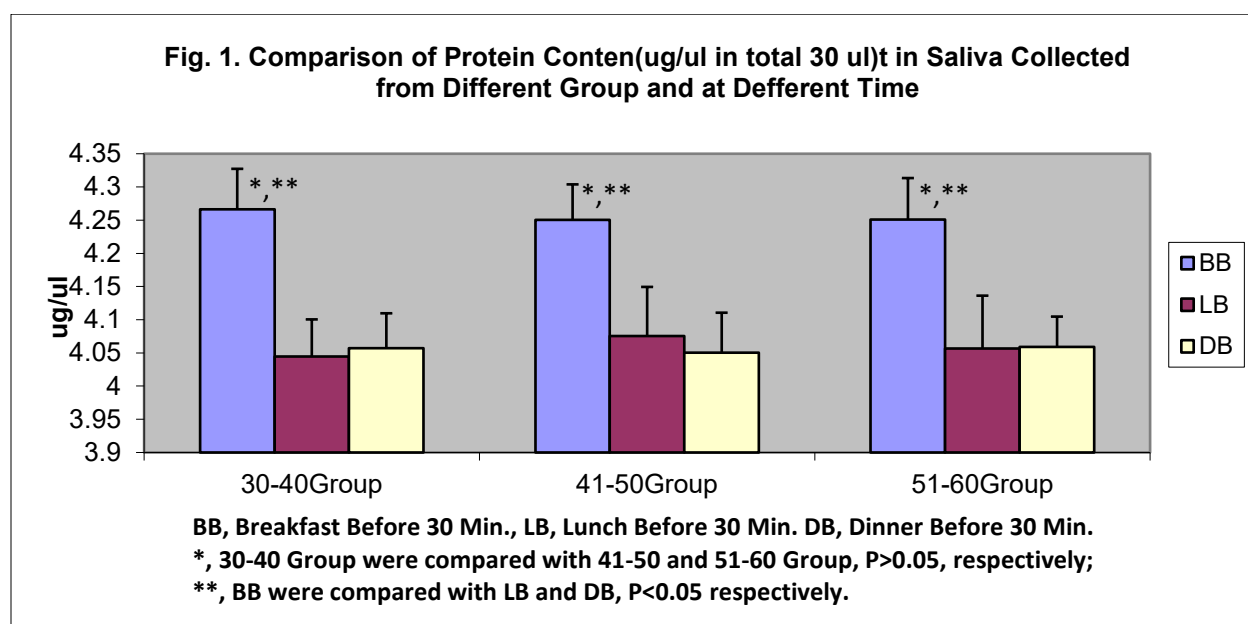
The saliva proteins extracted from each individual 200ul saliva were dissolved in 30 UL RIPA solution, and the protein was determined by ug/ul. The results were shown in table 1;

Table 1. ug/ul protein in 30 ul volume that extracted from 200 ul Saliva

	30-40 old years Group	41-50 old years Group	51- 60 old years Group
BB	4.27±0.06	4.25±0.05	4.25±0.04
LB	4.04±0.05	4.07±0.04	4.05±0.05
DB	4.07±0.04	4.06±0.06	4.06±0.03

BB, Breakfast before 30 min. LB, Lunch before 30 min. Dinner before 30 min.

3.2. Comparison of protein content in saliva collected at different age groups:



4. Discuss:

4.1. Saliva is a protein source on the study of protein levels in human diseases and health;

With the development of molecular biology, it has been proved that many diseases are caused by mutations in some genes⁵; However, the mutation of the gene site develops into a disease, first of all, the DNA is transcribed into RNA and then translated into protein by RNA, and the final result of the disease is determined by the function of protein expression⁶. At present, because there is not enough protein for the study of the disease, it is necessary to have a method to obtain enough protein to facilitate the study of disease⁷. This study can extract protein from saliva, can provide researchers obtain the dynamic body protein, the occurrence and development of disease to protein level; and studies to evaluate the human health status with the protein level of dynamic.

4.2. The extraction of salivary proteins will promote the research and development of protein biomarkers;

The study of biomarkers has made great progress in the diagnosis and treatment of many diseases⁸, However, the research on the development of human proteins and the development of protein biomarkers⁹. This study reports the extraction and extraction of saliva proteins, the availability of saliva to patients, and the researchers' easy access to proteins, which will have the potential to promote the research and progress of protein level biomarkers in the diagnosis and treatment of diseases.

4.3. Further research and development of salivary protein;

The study focused on saliva collection time and saliva protein extraction, but also proved that the highest protein content of saliva collected before

breakfast. However, the function of salivary proteins can be studied and the further research is planned.

5. Conclusion;

5.1. 200 UL saliva can obtain saliva protein 4.06-4.27ug/ul, in total 30ul protein extract;

5.2. Saliva protein content was highest in 30 minutes before breakfast;

5.3. Saliva collection and protein extraction are easy and convenient, which is the most convenient source of protein for the study on human diseases.

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