

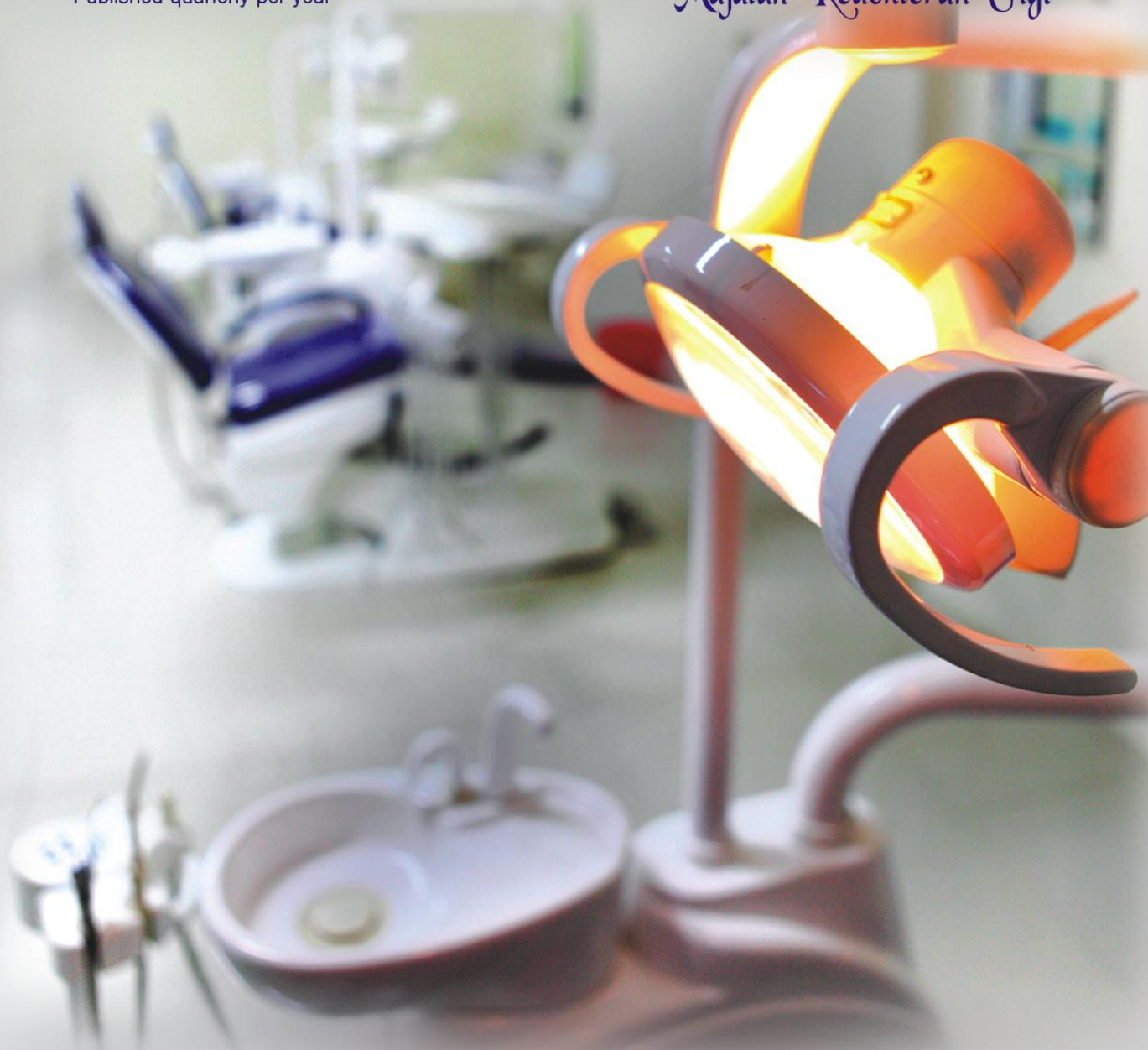
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- Endothelial cell cultured on HA/TCP/chitosan scaffold for bone tissue engineering
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Research Report

Elderly nutritional status effect on salivary anticandidal capacity against *Candida albicans*

Ria Puspitawati, Nurtami Soedarsono, Elisabeth A Putri, Anissha D Putri, and Boy M Bachtiar

Department of Oral Biology

Faculty of Dentistry, University of Indonesia

Jakarta - Indonesia

ABSTRACT

Background: Elderly often suffer malnutrition and oral candidiasis. *Candida albicans* (*C. albicans*) which is the most prominent cause of oral candidiasis, is one of commensal oral micro-flora. Nutritional status affects the characteristic of saliva. Saliva is the regulator in the development of *C. albicans* from commensal into pathogen. **Purpose:** The purpose of this study was to determine the correlation between elderly nutritional status with salivary total protein and its activity in inhibiting *C. albicans* growth and biofilm formation. **Methods:** Using mini nutritional assessment 30 elderly were classified into normal and malnutrition groups. Total protein of unstimulated saliva was measured using Bradford protein assay. The colony forming unit (CFU) of *C. albicans* was counted on 72 hours on SDA cultures without (control) or with 2 hour saliva exposure. Biofilm formation was analyzed from the optical density of 10^{-5} *C. albicans* suspension without saliva exposure (control) or with exposure of 10.000 µg/ml saliva and incubated in 37° C for 2 days. The suspension was put into 96 well plates, stained with crystal-violet dye, and analyzed using microplate reader. Differences between groups were analyzed using independent t-test or Kruskal-Wallis. Correlation between variables was analyzed using Spearman test. **Results:** Salivary total protein of normal elderly ($1.113.5 \pm 1.1143.3$) was higher than those of malnutrition (613.6 ± 253.6) but not statistically significant ($p > 0.05$). The CFU of *C. albicans* exposed to saliva of normal samples (2.060 cfu/ml) was significantly lower than control (24.100 cfu/ml) and those exposed to malnutrition saliva (5.513.3 cfu/ml). *C. albicans* biofilm formation is highest in controls (0.177), lower in those exposed to malnourished saliva (0.151) and lowest in those exposed to saliva of good nourished elderly (0.133). **Conclusion:** Although does not cause significant decrease of salivary total protein, malnutrition in elderly results in lower capacity of saliva in inhibiting the growth and declining the virulence of *C. albicans*.

Key words: *Candida albicans*, elderly, malnutrition, saliva

ABSTRAK

Latar belakang: Lansia sering menderita malnutrisi dan kandidiasis oral. *Candida albicans* yang merupakan penyebab utama terjadinya kandidiasis, adalah salah satu mikroflora rongga mulut yang bersifat konvensional. Malnutrisi memengaruhi karakteristik saliva. Saliva merupakan regulator utama perkembangan *Candida albicans* (*C. albicans*) dari sifat konvensional menjadi bersifat patogen. **Tujuan:** Tujuan dari penelitian ini adalah untuk menentukan korelasi antara status gizi lansia dengan total protein dan aktivitas saliva dalam menghambat pertumbuhan dan pembentukan biofilm *C. albicans*. **Metode:** Menggunakan mini nutritional assessment, 30 lansia diklasifikasikan menjadi kelompok gizi baik dan gizi buruk. Total protein unstimulated saliva diukur dengan metode Bradford protein assay. Colony forming unit (CFU) dihitung pada kultur *C. albicans* pada sabouraud dextrose agar (SDA) berusia 72 jam yang sebelumnya telah dipaparkan saliva selama 2 jam. Kontrol adalah kultur *C. albicans* tanpa paparan saliva. Pembentukan biofilm adalah pengukuran optical density suspensi 10^{-5} *C. albicans* tanpa paparan saliva (kontrol) atau dengan paparan saliva 10.000 µg/ml dan diinkubasi pada suhu 37° C selama 2 hari. Suspensi tersebut kemudian dimasukkan ke dalam 96 well plates, diberi pewarna crystal violet, dan diukur menggunakan microplate reader. Analisis data menggunakan uji beda t Independen atau Kruskal-Wallis, dan uji korelasi Spearman. **Hasil:** Total protein saliva lansia gizi baik ($1.113,5 \pm 1.1143,3$) lebih tinggi dari lansia gizi buruk ($613,6 \pm 253,6$) tetapi tidak bermakna secara statistik ($p > 0,05$). Pembentukan koloni *C. albicans* yang terpapar saliva lansia gizi baik (2.060 cfu/ml) secara signifikan lebih rendah dari kontrol (24.100 cfu/ml) dan daripada yang terpapar saliva lansia gizi buruk (5.513,3 cfu/ml). Pembentukan biofilm *C. albicans* tertinggi pada kontrol (0,177), lebih rendah pada

yang terpapar saliva gizi buruk (0,151) dan terendah pada yang terpapar saliva gizi baik (0,133). **Kesimpulan:** Meskipun malnutrisi tidak menyebabkan penurunan total protein saliva lansia, tetapi menurunkan kapasitasnya dalam menghambat pertumbuhan dan mengurangi virulensi *C. albicans*.

Kata kunci: *Candida albicans*, lansia, malnutrisi, saliva

Correspondence: Ria Puspitawati, c/o: Departemen Biologi Oral, Fakultas Kedokteran Gigi Universitas Indonesia. Jl. Salemba Raya No. 4 Jakarta Pusat, Indonesia. E-mail: riapuspitawati@yahoo.com

INTRODUCTION

Elderly is prone to malnutrition mainly due to three ethiological factors. Firstly, medical factors such as decreased appetite, malabsorption, disturbances of oro-dental tissue, taste, and smell sensation, presence of infection, systemic disturbances, and medicine side effects. Second factor includes social condition such as poverty, social isolation, and decreased ability to provide meal. Third factor is the psychosocial factors such as depression and dementia.^{1,2} Disturbances of nutrition intake will eventually lead to alteration of nutritional status. Poor nutrition in elderly could result in decreased ability in responding to any physiological challenge and decreased immunity which will be reflected as susceptibility in healing process and in resistance to infection.²

Malnutrition results in decreased ability of immune system in protecting oral tissue from infection. Oral infection commonly suffered by elderly is candidiasis. Common predisposing factors of oral candidiasis in elderly include decreased immunity, presence of chronic disease, long term antibiotic intake, poor oral hygiene, decreased salivary flow rate, and malnutrition.^{3,4} Presence of denture is another common condition in elderly which could trigger the occurrence of oral candidiasis. A significant correlation between malnutrition and oral candidiasis was reported.⁵ The presence of oral candidiasis usually causes annoying pain which disturbs mastication function and aggravate the existed malnutrition.^{6,7}

Candida albicans (*C. albicans*) which is the most prominent cause of oral candidiasis, is one of commensal oral micro-flora. The behaviour and expression of this yeast is correlated to decreased immune system of the host, as found in elderly and malnutrition subject.^{5,8,9} Besides the equilibrium between the yeast and other oral microbes, *C. albicans* distribution and virulence in oral cavity are also influenced by its adhesion capability, proteolytic enzymes production, and hyphal formation.^{10,11} One defence mechanism of *Candida* spp. against the anti *Candida* components of immune system in saliva is biofilm formation. The development of biofilm is related to the aggravation of clinical infection on the host cell. In oral cavity, *C. albicans* could adhere and form biofilm on the surfaces of dental and oral mucous tissue or on denture base.^{11,12}

In oral cavity, saliva has role in affecting the existence, composition, and behaviour of the microflora via clearance

effect mechanism. In such mechanism, saliva mediating the activity of molecules responsible as the anti-microorganism or anti fungal of oral immune system.¹³ Saliva is the main regulator in the development of *C. albicans* from commensal into pathogen. Such regulatory function is especially determined by the salivary protein composition. Salivary proteins known to have anti-candida capacity are sIgA, defensin, histatin, lactoferrin, lysozyme, and mucin.^{10,14,15} Adequate nutrient intake influencing the quantity and the biological activity of saliva and affect its effectiveness as ecological equilibrium regulator in oral cavity.¹⁶ Various previous studies reported decreased protein components concentration of saliva in protein-calorie malnutrition cases.^{2,9,15-17}

Among two methods commonly used in measuring protein concentration, Bradford assay is considered to be the simpler (could be read in 5 minutes) and more sensitive method compared to Lowry method. In Bradford protein assay, the concentration of protein measured is based on the formation of complex formed by brilliant blue dye and protein in the sample. The presence of protein-dye complex results in alteration of maximum absorption of the dye when read using 465–595 nm absorbance length. The determination of total protein concentration of sample is based on the comparison with the concentration of standard protein (bovine serum albumin).¹⁸

The objective of this study was to determine the correlation between elderly nutritional status with salivary total protein and its activity in inhibiting *C. albicans* growth and biofilm formation.

MATERIALS AND METHODS

Subject of this study were elderly aged 65–80 who did not consume antibiotic/anti virus during the last 3 months, not having history of DM, HIV infection/AIDS, or malignancy, able to communicate, and willing to participate in the project by signing the informed consent.

Nutritional status of the samples were determined by using mini nutritional assessment (MNA) developed by NESTLE. From this assessment subjects were classified into poor nutrition (< 18.5) and good nutrition (18.5–25) groups.

Fifteen milliliters unstimulated saliva was collected from each subject, placed in cooled closed tubes, centrifuged at 10.000 rpm for 5 minutes. The supernatant was kept in

–20° C until used. Total protein of sample’s saliva was analyzed using Bradford protein assay and read using microplate reader at 490 nm absorbance wave length.¹⁸

C. albicans strain ATCC 10231 obtained from Dept. Microbiology, Faculty of Medicine, Universitas Indonesia was used in this study. *C. albicans* was cultured in SDA at room temperature for 72 hours, and diluted into 10⁻³ suspension.

The inhibition effect of saliva against *C. albicans* growth was analyzed by counting the colony forming unit (CFU) of 10⁻³ *C. albicans* suspension cultured on sabraud dextrose agar (SDA) for 72 hours at room temperature. Comparison were made between the CFU of *C. albicans* which previously been exposed to saliva of good-nutrition elderly, or to saliva of poor-nutrition elderly, or not exposed to saliva (control). Saliva exposure on *C. albicans* was conducted by mixing 10 µl of 10⁻³ *C. albicans* suspension with 50 µl of 10.000 µg/ml diluted saliva, and incubated in 37° C waterbath. After 2 hours the mixture was diluted to make 10⁻³ solution from which 10 µl was cultured on SDA at room temperature for 72 hours.

The inhibition effect of saliva against *C. albicans* biofilm formation was analyzed by measuring the optical density of 10⁻⁵ *C. albicans* suspension which previously been exposed to 10.000 µg/ml saliva and incubated in 37° C for 2 days. The suspension was put into 96 well plates, stained with 10% crystal violet, and read using microplate reader with 655nm wave-length, following method used by Paramanova¹⁰ modified by method used by Bastiaan.¹⁹

Differences between groups were analyzed using Independent t-test or Kruskal-Wallis, while correlation between variables was analyzed using Spearman test. Degree of confidence was 0.05.

RESULTS

From 30 samples used in this study, the MNA score of 15 subjects in good nutrition group were 20–24.5, while the MNA score of 15 subjects in poor nutrition group were 15.5–18.5. Nine out of 15 subjects in poor nutrition group have MNA score between 17–18.5.

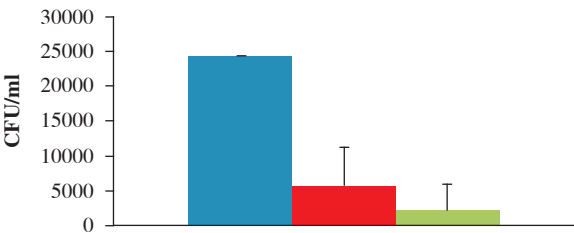


Figure 1. *C. albicans* CFU after exposed to elderly saliva with different nutritional status on the growth of *C. albicans*. Blue = control, Red = Poor nutrition, Green = Good nutrition.

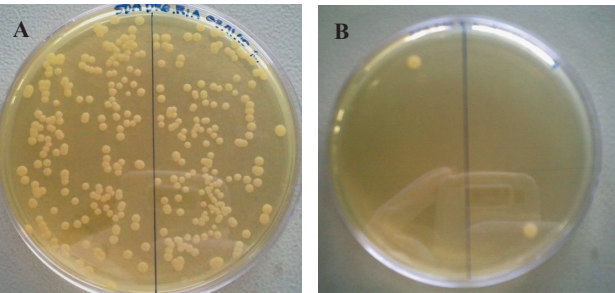


Figure 2. *C. albicans* cultures on medium exposed to saliva of elderly with poor nutritional status (a) and good nutritional status (b).

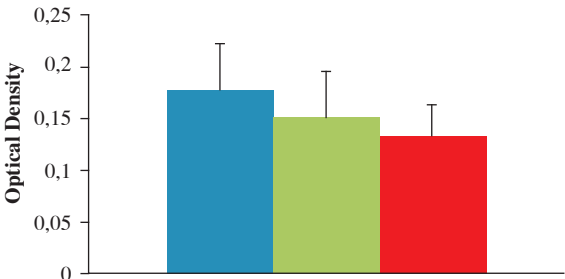


Figure 3. *C. albicans* biofilm formation of cultures not exposed to saliva (control), exposed to saliva from poor nutrition elderly, and from good nutrition elderly. Blue = control, Green = poor nutrition, Red = Good nutrition.

Comparison of salivary total protein concentration between groups was analyzed using independent t test while correlation between elderly nutritional status and salivary total protein was analyzed using Pearson test. As could be seen in table 1, mean of saliva total protein concentration for good nutrition group is higher than those for poor nutrition group. However, this difference is not statistically significant ($p > 0.05$). Pearson correlation test revealed that there is a weak ($r = 0.323$) positive correlation between elderly nutritional status and salivary total protein. This means that higher nutritional status relevance to higher salivary total protein concentration. However, this correlation is not statistically significant ($p > 0.05$).

The parameter for *C. albicans* growth in this study is the CFU of the yeast on SDA medium. As could be seen in graph 1, the CFU of control *C. albicans* (not exposed to saliva) is prominently higher than those exposed to saliva, either of those exposed to saliva from good or from poor nutrition group. The CFU of *C. albicans* exposed to saliva of good nutrition elderly was also lower than those exposed to saliva of poor nutrition elderly (Figure 2a,b). Kruskal-Wallis test revealed that all these differences were significant, $p < 0.05$.

Spearman correlation test showed there is a weak ($r = 0.395$) negative correlation between elderly nutritional status and *C. albicans* growth. This means that higher nutritional status

Table 1. Saliva total protein concentration difference between groups and correlation between elderly nutritional status and saliva total protein concentration

Groups	N	Saliva total protein Mean (mg/ml) ± SD	p (T test)	p (correlation)	r	Correlation direction
Good nutrition	14	1113.55 ± 1143.3	0.111	0.093	0.323	Positive
Poor nutrition	15	613.60 ± 253.60				

is relevant to lower growth of *C. albicans*. Figure 1 shows the pronounced difference of *C. albicans* CFU between those exposed to good nutrition and those exposed to poor nutrition elderly.

Kruskall-Wallis test confirmed that the formation of *C. albicans* biofilm on *C. albicans* culture not exposed to saliva is significantly higher than those exposed to saliva (either from good or poor-nutrition elderly). Conversely, the biofilm formation on medium exposed to saliva from poor nutrition elderly is not significantly ($p > 0.05$) higher than those exposed to saliva from good nutrition elderly (Figure 3).

Pearson correlation test confirmed that a weak ($r = 0.243$) negative correlation between elderly nutritional status and *C. albicans* biofilm formation is not significant ($p > 0.05$). There is a tendention that the lower nutritional status relevance to higher *C. albicans* biofilm formation.

DISCUSSION

Relevance to previous studies that decreased concentration of salivary total protein could be due to aging, poor nutrition, and disturbances of salivary gland,²⁰ result of this study also showed pattern of lower salivary total protein concentration on poor nutrition elderly compared to those with good nutritional status. However, such differences were not statistically significant. Such results could be due to composition of the samples where 60% subjects (9 out of 15) in poor nutrition group were actually in borderline nutritional status. MNA is simple, has 98% accuracy, and frequently used to determine the nutritional status of elderly. In MNA method developed by NESTLE, nutritional status is classified as good (> 18.5), moderate (17–18.5), and poor (< 17). The borderline status indicates condition with high risk to suffer malnutrition and not yet actually in malnutrition condition.²¹

Another possible reason for results of this study was that slight difference of nutritional status of elderly participated in this study, does not lead to different concentration of their salivary total protein. Such result is similar to a previous study by Johansson *et al.*,²² who reported indifferent secreted total protein per minute between control and sample with protein-energy-malnutrition (PEM).

This study provide evidence of the efficacy of saliva in inhibitting the growth of *C. albicans*. As could be seen in figure 1, even saliva from elderly still has the capacity to inhibit the colony formation of *C. albicans* indicating

inhibition of the yeast growth provided that the nutritional status of the subject is adequate. The pattern of decreased total protein concentration following decreased nutritional status, is relevant with the pattern of weaken inhibitting effect of saliva on the growth of *C. albicans* following decreased nutritional status. Saliva contains many proteins with anti candidal capacity. Malnutrition might lead to either lower secretion²³ or weaker activity of these proteins.²⁴ It is known that not all salivary proteins are secreted less in response to malnutrition. Lisozim, lactoferrin and sIgA are three among various salivary proteins which were reported to decrease in children with PEM.^{7–9,15,16} Further study is required to determine the correlation between elderly nutritional status with concentration of each salivary antifungal protein. However, insignificant correlation between salivary total protein and elderly nutritional status found in this study indicating that nutritional status affects the salivary effectivity against *C. albicans* stronger than it affects the salivary protein concentration.

This study also confirmed the efficacy of saliva, even the saliva of elderly with poor nutritional status, in inhibitting *C. albicans* biofilm formation. Saliva has double roles in host-*Candida* interaction. Not only providing water, nutrition, and anti fungal components for the host, saliva also provides support for candida adhesion on the oral tissue surfaces.²⁵ However, results of this study revealed that the role of saliva as anti fungal should be stronger than its regulatory role in supporting the adhesion of *Candida*. The insignificant efectivity of saliva in ihibitting *C. albicans* biofilm formation between elderly with different nutritional status might be due to the composition of nutritional status of samples used in this study.

Although elderly nutritional status does not lead to significant decreased salivary total protein concentration but it is significantly correlated to salivary anti candidal acitvity against *C. albicans*. Poor nutritional status which has not affected the quantity of salivary total protein might already lead to weaken effectivity of saliva against *C. albicans*. In conclusion, nutritional status of elderly affect the capacity of saliva both in inhibiting the growth and in declining the virulence of *C. albicans*.

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