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EVALUATING SALIVARY α - AMYLASE LEVELS FOLLOWING TRIGONA SP HONEY APPLICATION TO POST PALATOPLASTY PATIENTS: A PILOT RANDOMIZED CLINICAL STUDY

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ABSTRACT

Background. Palatoplasty aims to restore palatal continuity and improve velopharyngeal function. Post-palatoplasty wound healing may affect tissue quality, scar tissue formation, and upper jaw growth. Trigona sp. honey and framycetin sulfate are used to inhibit bacterial colonization and accelerate wound healing. Buccal fat pads can increase tissue vascularization and accelerate wound epithelialization in the donor site. α-amylase is a biomarker used to evaluate the wound healing process and to monitor the inflammatory response. This study evaluated salivary α-amylase level as a marker of wound healing following Trigona sp. honey and framycetin sulfate with or without buccal fat pad in post-palataloplasty patients. **Methods**. A pilot randomized clinical study was conducted to evaluate α-amylase levels in the four treatment groups measured preoperatively and on the fourth and seventh days postoperatively. Sample size estimation using G*Power version 3.1 determined a minimum of 24 participants, and statistical analysis was performed using IBM SPSS version 25. **Results**. The α-amylase level in the framycetin sulfate dressing with buccal fat pad was higher than that in Trigona sp. honey with buccal fat pad, but the difference in α-amylase levels was not significant. Trigona sp. honey can serve as an effective and economical alternative to wound dressings. **Conclusion**. α-amylase levels changed in all treatment groups, increasing on the fourth day postoperatively and decreasing on the seventh day. Framycetin sulfate dressing with buccal fat pad application was more effective than Trigona sp. honey with buccal fat pad application, but the difference in α-amylase levels was not significant.

Keywords: buccal fat pad, cleft palate, framycetin sulfate, honey, salivary alpha-amylase

1. INTRODUCTION

Palatoplasty is a surgical procedure to repair a cleft palate, aimed at improving facial appearance, enhancing swallowing function, aiding speech development, and reducing the psychological impact on patients.[1,2] The management of cleft palate is an inherently complex process and requires a coordinated multidisciplinary approach.[3–5] Palatoplasty can result in scar formation, which may interfere with upper jaw growth and dental development.[6]

Palatoplasty will cause injury, which will occur in the wound healing process through four phases: hemostasis, inflammation, proliferation, and remodeling.[1] Key factors in wound healing include positive regulators and the stimulation of angiogenesis mechanisms, which significantly influence the repair of the epidermis, granulation tissue, and the formation of high-quality tissue remodeling.[7] Rapid wound closure can reduce the risk of infection and lower morbidity rates in patients. If the wound healing process is delayed, the wound may become chronic.[8]

In general, edema, inflammation, and exudation, which are common in all types of wounds, can be reduced with honey to enhance wound healing.[9,10] Honey has been used as a remedy for various types of wound healing and is a safe food product for consumption, making it suitable for intraoral application.[10] Trigona sp. honey contains higher levels of flavonoids, phenolics, and antioxidants than several other types of honey.[10] These antioxidant compounds play a role in inhibiting the formation of free radicals produced during the tissue inflammatory process with reduced oxidative stress, tissue damage can be minimized, allowing the tissue regeneration process to proceed more rapidly and stably.[11,12]

The dressing commonly used in palatoplasty is framycetin sulfate, an aminoglycoside antibiotic that works by inhibiting bacterial protein synthesis.[13] Microorganisms sensitive to framycetin sulfate include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and some strains of *Pseudomonas aeruginosa*. Framycetin sulfate has been shown to be one of the optimal dressings for reducing bacteria on the wound surface and accelerating the healing process.[13,14]

The buccal fat pad is a complex structure associated with important anatomical structures such as the facial nerve, parotid gland, and masticatory muscles.[15,16] The buccal fat pad is recognized as a practical and beneficial flap for the repair of primary cleft palate, particularly in enhancing mucosal healing, reducing the risk of complications, and improving functional outcomes such as speech development and upper jaw growth.[15,17]

The inflammatory reaction stimulates salivary secretion and increases salivary α-amylase levels, along with increasing the speed of saliva flow.[18] One of the saliva proteins is α-amylase, which can serve as an effective, economical, and non-invasive biomarker for evaluating inflammation during the wound healing process.[18] Saliva is considered a diagnostic

tool that can provide molecular biomarkers for various oral and systemic diseases.[19] Saliva contains various proteins, hormones, antibodies, medications, and cytokines.[19]

Based on the above background, saliva biomarkers can be considered diagnostic tools for wound healing, and honey has beneficial effects on the wound healing process. This article aims of study aimed to evaluate salivary α-amylase levels as a marker of wound healing following Trigona sp. honey administration in post-palataloplasty patients.

2. MATERIALS AND METHOD

2.1 Study design

This pilot randomized clinical study evaluated α-amylase levels in four treatment groups measured preoperatively, on the fourth and seventh days postoperatively at Hasanuddin University Dental Hospital and HUM-RC Laboratory, Hasanuddin University Hospital, Makassar, Indonesia. Ethical approval was obtained from the Ethics and Research Committee of the Faculty of Dentistry, Hasanuddin University (No. 074/KEPK FKG-RSGMP UH/EA/III/2025) and was performed according to the Consolidated Standards of Registered Trials (CONSORT) guidelines.

2.1.1 Sample size calculation

The literature on randomized clinical pilot studies in children with cleft palates was surveyed to determine an appropriate sample size range. To the best of our knowledge, no other study has evaluated α-amylase as a marker of wound healing using *Trigona sp.* honey in children with cleft palate.

Sample size estimation was performed using G*Power version 3.1 (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) for repeated-measures ANOVA with a within-between interaction. Assuming a medium effect size (f = 0.25), α error probability of 0.05, statistical power of 80%, two groups, and repeated postoperative measurements, the minimum required sample size was calculated as 24 participants.

2.1.2 Eligibility criteria

Eligible participants were patients with a diagnosis of unilateral cleft palate aged 18 months to 6,5 years. Patients undergoing palatoplasty surgery between June 2024 and June 2025 at Hasanuddin University Dental Hospital and Celebes Cleft Centre Foundation, Makassar, Indonesia, and all participants parents agreed and signed an informed consent form to participate in examinations and evaluations through to the end of the study in accordance with research procedures. The exclusion criteria were a history of systemic disease, allergies to honey ingredients, and failure to follow the examination and evaluation until the end according to the research procedure.

2.1.3 Randomization, allocation concealment and blinding

The 24 patients undergoing palatoplasty were randomly allocated into four treatment

groups: (1) Trigona sp. honey dressing with the buccal fat pad technique, (2) Trigona sp. honey dressing without the buccal fat pad technique, (3) framycetin sulfate dressing with the buccal fat pad technique, and (4) framycetin sulfate dressing without the buccal fat pad technique. The randomization sequence was generated using random numbers provided by a laboratory technician. Allocation concealment was ensured using Eppendorf tubes numbered sequentially. Owing to the nature of the intervention, operator blinding was not feasible; the study can be described as single-blinded, with the laboratory technician performing the saliva analysis not involved in the surgical procedure or patient care.

2.2 Surgical procedure

All patients underwent palatoplasty using the two-flap push-back technique under general anesthesia. Palatoplasty surgery was performed trigona sp honey dressing with or without the use of the buccal fat pad technique and framicetyn sulfate dressing with or without the use of the buccal fat pad technique. In the intervention group, after oral mucosa sutures, the buccal fat pad was retracted and sutured to the lateral defect. Next, in the Trigona sp. honey dressing group, sterile gauze impregnated with Trigona sp. honey or framicetyn sulfate was applied according to the size of the obturator to be fixed.

2.3 Preparation and application of the Trigona sp. honey and Framycetin sulfate dressing

During the operation, after oral mucosal sutures, the amount of honey applied was ± 5 ml with a note: the condition of the honey absorbs all sides of the sterile gauze, then fixed with an obturator. Meanwhile, the framicetyn sulfate dressing was fixed with an obturator in the post-palastoplasty area.

2.4 Clinical Follow-up

Clinical follow-up was performed on the fourth and seventh days postoperatively. After the removal of the obturator, the intraoral condition revealed no blood clots or active bleeding, with minimal hyperemia. The intraoral condition at one month after surgery showed that the lesions in the surgical area were well characterized by no dehiscence of the palatal fistula.

2.5 Outcome measure and saliva sampling

Preoperative and postoperative (on the fourth and seventh days) α-amylase levels were measured in the four groups. One milliliter of saliva was collected using an aspiration pipette and stored in an Eppendorf tube and can be stored in the refrigerator at a temperature of -20^0 to -80^0 until all samples are collected and are ready for analysis in the laboratory. Salivary analysis was performed using a Human AMY1 Alpha-1 Amylase, Salivary ELISA Kit (Elabscience, Houston, Texas, USA). The main comparison of interest was the difference in salivary α-amylase activity between Trigona sp honey (Hasanuddin University, Indonesia) dressing with or without the buccal fat pad technique and framycetin sulfate (Darya-Varia Laboratory, Gunung Putri, Bogor, Indonesia) dressing with or without the buccal fat pad

technique, and the results showed an effective sample group.

2.6 Statistical analysis

Data analysis will be performed using IBM SPSS version 25 (IBM Corp., Armonk, NY, USA). The subject characteristics will be analyzed descriptively. In this univariate analysis, a description was also provided to assess the characteristics of each variable analyzed in this study. The analysis will then proceed to a bivariate analysis to examine differences in saliva values (α-amylase) at each specified time point across the four treatment groups. The research data will then undergo a Shapiro-Wilk normality test with a significance level of $p < 0.05$, as the sample size is ≤ 50 (24 samples). Homogeneity was tested using Levene's test of variance with a significance level of ($p > 0.05$). The bivariate analysis used in this study, assuming normally distributed data, was mixed ANOVA. Subsequently, a Tukey's post hoc test was performed to identify the groups that differed in the mixed ANOVA.

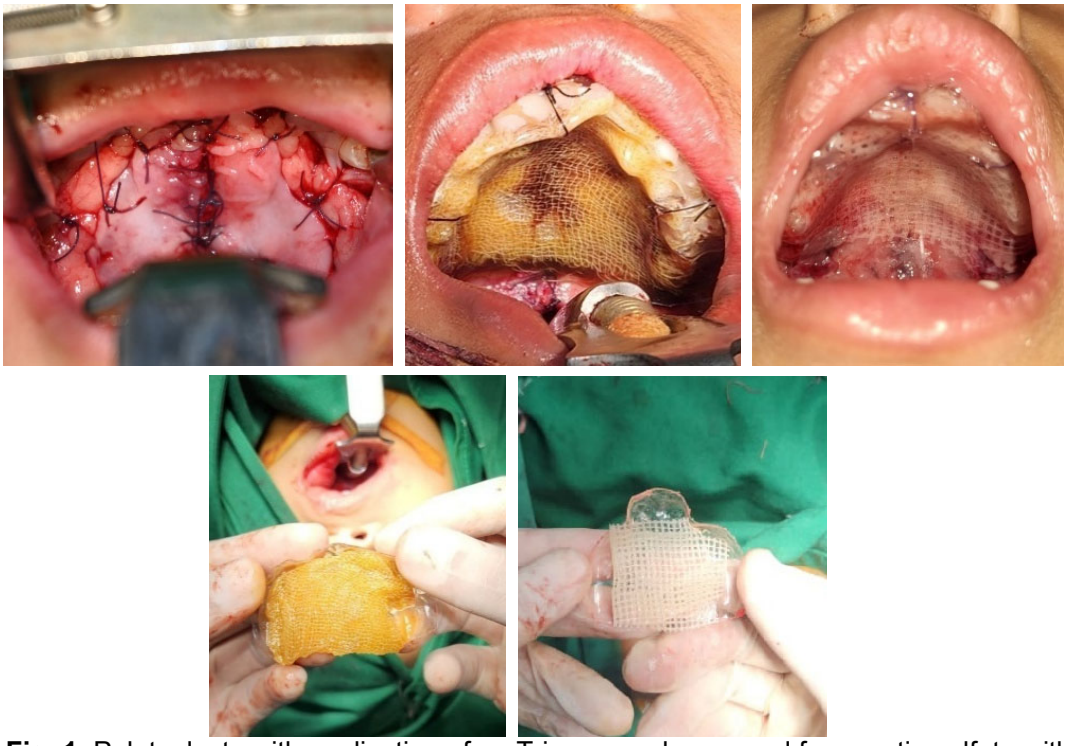

Fig. 1. Palatoplasty with application of an Trigona sp. honey and framycetin sulfate with or without buccal fat pad.

3. RESULTS

This study included six female and 18 male patients in the 18-month- to 6.5-year age group. Based on the type of cleft palate, the patients were divided into eight patients with Veau Type II cleft palate and 16 patients with Veau Type III cleft palate with the two-flap pushback technique in the group treated with Trigona sp. honey dressing with or without the buccal fat pad technique and framycetin sulfate dressing with or without the buccal fat pad technique (Table 1).

Table 1. Basic Characteristics of Research Sample

Variabel	Research Sample	
	N	%
Gender		
Male	18	75%
Female	6	25%
Cleft Palate Classifications		
Type II (Veau Classification)	8	33,3%
Type III (Veau Classification)	16	66,7%
Intervention		
- Trigona sp honey dressing with buccal fat pad technique	6	25%
- Trigona sp honey dressing without buccal fat pad technique	6	25%
- *Framycetin sulfate dressing with buccal fat pad technique*	6	25%
- *Framycetin sulfate dressing without buccal fat pad technique*	6	25%

A comparison of changes in α-amylase levels before and after palatoplasty showed an increase on the fourth day and a decrease on the seventh day post-surgery. The framicetyn sulfate without the buccal fat pad technique dressing group exhibited the highest increase on the fourth day, and the trigona sp honey group with the buccal fat pad technique experienced the highest decrease in results on the seventh day after surgery. The framycetin sulfate dressing group with the buccal fat pad technique showed the lowest increase on the fourth day and the trigona sp honey without the buccal fat pad technique group had the lowest decrease in results on the seventh day after surgery. (Table 2).

Table 2. Mean values and standard deviations (α-amylase) pre and post palatoplasty

Sample Group	Average ± SD Value (α-Amylase)		
	Preoperatively	Postoperatively day-4	Postoperatively day-7
Trigona sp honey dressing with buccal fat pad technique	0.827±0.196	1.753±0.3	1.308±0.298
Trigona sp honey dressing without buccal fat pad technique	**1.024** ±0.325	3.605±0.515	2.361±0.489
Framycetin sulfate dressing with buccal fat pad technique	0.867±0.194	1.668±0.2	1.325±0.184
Framycetin sulfate dressing without buccal fat pad technique	0.808±0.103	3.68±0.463	1.842±0.341

The Tukey post hoc analysis results showed that for Trigona sp. honey + buccal fat pad versus framycetin sulfate dressing + buccal fat pad and for Trigona sp. honey versus framycetin sulfate, the p-value was >0.05; therefore, there was no significant difference in the α-amylase levels between these samples. Trigona sp. honey + buccal fat pad compared to

Trigonasp. honey and framycetin sulfate, and framycetin sulfate dressing with buccal fat pad compared to Trigonasp. honey and framycetin sulfate yielded p-values <0.05; therefore, it was concluded that there were significant differences in these samples based on the α-amylase values.

Table 3. Results of Tukey's Post Hoc Analysis

Sample Group	Sample Group	p
Trigona sp honey dressing with buccal fat pad technique	Trigona sp honey dressing without buccal fat pad technique	.000
	Framycetin sulfate dressing with buccal fat pad technique	1.000
	Framycetin sulfate dressing without buccal fat pad technique	.000
Trigona sp honey dressing without buccal fat pad technique	*Framycetin sulfate dressing with buccal fat pad technique*	.000
	Framycetin sulfate dressing without buccal fat pad technique	.523
Framycetin sulfate dressing with buccal fat pad technique	*Framycetin sulfate dressing without buccal fat pad technique*	.000

Clinical evaluation revealed no adverse events or postoperative complications associated with the topical application of Trigona sp. honey. Throughout the follow-up period, the participants showed no signs of allergic reactions, secondary infections, wound dehiscence, excessive bleeding, or hypertrophic scarring. This treatment was well tolerated by all patients, with α-amylase levels reflecting a physiological healing process free of systemic inflammatory disturbances. These findings suggest that Trigona sp. honey functions as a biocompatible material for palatoplasty, offering a favorable safety profile for clinical use.

4. DISCUSSION

The results of the study showed that all treatment groups exhibited a similar pattern of change in α-amylase levels, namely an increase on the fourth day post-surgery, followed by a decrease on the seventh day. Physiologically, this increase reflects an acute stress and inflammatory response resulting from surgical trauma.[20] Salivary α-amylase is a biomarker of sympathetic nervous system activity that increases under conditions of stress, inflammation, and tissue pain.[21]

An increase in α-amylase on the 4th day postoperatively can be interpreted as a manifestation of an acute inflammatory phase that occurs in the first 24-48 hours characterized by the infiltration of neutrophils and macrophages as well as the release of inflammatory mediators.[20,22] In this phase, the infiltration of neutrophils and macrophages into the wound area occurs, followed by the release of inflammatory mediators such as interleukin, tumor necrosis factor-α, and various other proinflammatory cytokines. This inflammatory response

plays a role in clearing necrotic tissues and preparing the wound environment for the subsequent tissue regeneration process.[23]

The decrease in α-amylase levels on the 7th postoperative day indicates that the inflammatory process is beginning to subside and that the tissue is entering the proliferation phase. During this phase, fibroblast activation, granulation tissue formation, angiogenesis, and wound epithelialization occur.[1] The decrease in inflammatory biomarkers signifies the stabilization of the body's physiological response to the surgical trauma. Therefore, changes in α-amylase levels in this study may reflect the biological dynamics of the systemic healing process of palatal mucosal wounds.[19]

A clinical study conducted by Zhafirah et al[1], the role of forest honey in accelerating wound healing in a palatoplasty animal model, specifically its ability of forest honey to increase VEGF protein expression. In silico analysis indicated the role of genistein in forest honey that affects the cell's angiogenesis and proliferation mechanism. In a case report, David et al[20] demonstrated the intraoral condition revealed no blood clots and active bleeding with minimal hyperemia. The intraoral condition at one month after surgery showed uneventful healing.

Ahmed et al[13] described that betamethasone + polymyxin B dressing is better in the STSG donor area than sterile tulle graft dressings with 1% framycetin sulfate. Betamethasone + polymyxin healed more effectively than the other preparations because of a short healing period, mild pain, and the lowest rate of infection or scarring. Both are credited for their anti-inflammatory and antimicrobial effects, which accelerate epithelialization of the wound. In this study, framycetin sulfate dressings applied using the buccal fat pad technique were more effective than Trigona sp. honey applied using the buccal fat pad technique; however, the difference in α-amylase levels was not highly significant.[11]

The results of this study have important clinical implications for oral and maxillofacial surgical practice, particularly in the management of post-palatal wounds. The use of Trigona sp. honey as a wound dressing can serve as an effective and economical alternative; honey is a natural substance that is relatively safe, readily available, and possesses broad therapeutic effects in the wound healing process.[20] The use of salivary biomarkers, such as α-amylase, also offers a new approach to non-invasively monitor the wound healing process. Saliva testing is relatively easy to perform, causes no pain to the patient, and can provide information regarding the body's physiological response to wound healing.[24]

This study had several limitations that should be considered when interpreting the results. The use of α-amylase as a single biomarker cannot represent the complexity of the wound healing process, which involves various biological mediators, such as inflammatory cytokines, growth factors, and extracellular matrix proteins. Additionally, methodological

limitations include a small sample size, non-random subject allocation, and technical challenges in collecting saliva from pediatric patients, such as flow-rate variability, low patient cooperation, and the risk of contamination from oral debris and gingival microbleeding, which could potentially trigger reactivity and bias in the α-amylase results. Therefore, future research should combine multiple molecular biomarkers to obtain a more comprehensive understanding of wound healing mechanisms and explore specific non-invasive clinical indicators to support broader clinical implementation.

5. CONCLUSION

In this study, we found that all treatment groups experienced a similar pattern of changes in α-amylase values, namely an increase on the fourth postoperative day and then a decrease on the seventh day. In this study, framycetin sulfate dressing with the buccal fat pad technique was more effective than Trigona sp. honey with the buccal fat pad technique; however, the difference in α-amylase levels was not significant.

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DECLARATION OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ETHIC APPROVAL

This study was approved by the Ethics Commission of the Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia (code number: 074/KEPK FKG-RSGMP UH/EA/III/2025).

AUTHORS' CONTRIBUTIONS

Nia Nurhaeni: Methodology, Investigation, resources, formal analysis, writing – original draft. Nisrina Ekayani Nasrun: Methodology, resources, formal analysis, supervision, writing–original draft. Eka Prasetiawaty: conceptualization, methodology, formal analysis, resources, supervision, writing–review & editing. Abul Fauzi: Conceptualization, methodology, investigation, resources, formal analysis, supervision, writing–original draft.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author

upon reasonable request.

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