Original Article

Caries Activity and Ph Level Changes after Fluoride Varnish and Casein Phosphopeptides-Amorphous Calcium Phosphate Application on Children's Saliva

Anie Apriani[1,2], Armelia Sari Widyarman[3], E. Arlia Budiyanti[4], Boedi Oetomo Roeslan[5]

[1]Post Graduate Student Faculty of Dentistry Trisakti University, Jakarta, [2]Department of Pediatric Dentistry, Maranatha Christian University, Bandung, [3]Department Head of Microbiology, Faculty of Dentistry, Trisakti University, Jakarta, [4]Department of Pediatric Dentistry, Faculty of Dentistry, Trisakti University, Jakarta, [5]Department of Oral Biology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia

Abstract

Background: Caries is a disease affecting the hard tissue of the tooth wherein the demineralization process caused by *Streptococcus mutans* decreases saliva pH faster than the remineralization process can maintain it. Topical fluoridation, such as fluoride varnish and casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) is the most common preventive therapy for the disease. **Aims:** The aim of this study is to assess the difference between fluoride varnish and CPP-ACP in reducing saliva pH and caries activity. **Materials and Methods:** This is an experimental study with a sample population of 60 children (aged 8–9 years old), divided into two groups of 30. Group 1 was treated with fluoride varnish, Group 2 with CPP-ACP. A *t*-test was used to measure the effects of the different treatments. **Results:** The result showed that the average difference in saliva pH before and after application was −0.12933 in Group 1 and −0.14033 in Group 2 (P = 0.256). The average difference in caries activity before and after application was 3.189 log colony-forming units (CFUs)/mL in Group 1 and 2.237 log CFUs/mL in Group 2 (P = 0.275). **Conclusion:** The most effective treatment for increasing saliva pH and reducing caries activity can be achieved by using the varnish for 1 month. However, there is no difference between fluoride varnish and CPP-ACP with regard to altering saliva pH and reducing caries activity statistically. Future study is needed to explore this result.

Keywords: *Caries activity, casein phosphopeptides-amorphous calcium phosphate, fluoride varnish, saliva pH*

Introduction

The epidemiology of tooth caries indicates that the problem is major and remains a problem over time.[1] It is a progressive dissolution of the inorganic components comprising the hard tissue of the tooth and attached media. The destruction of the hard tissue of the tooth is caused by a number of interacting factors which together cause caries.[2] Cariogenic bacteria demineralize and break down the hard tissue of a tooth faster than the remineralization process can maintain it. *Streptococcus mutans* is the most cariogenic bacteria involved in caries,[3,4] and the more acidic saliva is the more vulnerable it makes tooth tissue to caries. An individual's saliva can become more acidic because of its flow, viscosity, and pH.[5] Saliva also plays a main role in caries because it is an environment for bacterial activity,[6] working to clean tooth surfaces, dissolve food, lubricate the mouth, protect oral mucosa, and buffer effect.[7] A normal saliva pH is 5.75–6.5; a lower pH indicates that a patient's oral cavity is acidic due to a lack of oral hygiene, caries, calculus accumulation, or an inappropriate diet.[8] Saliva pH tests can predict causative bacterial activity.[9]

The oral and dental health report produced by Maranatha University's Faculty of Dentistry, Indonesia in 2016 included a sample population of 260 children aged 6–7 years old children; of these, 246 (94.6%) were positive for tooth caries and the caries index was 8.18 in the Sukajadi sub-district of Bandung, Indonesia. They were treated with topical fluoride applied via a mouth rinse, however, this treatment was failed to decrease the caries index. The next examination in the following year (2017) revealed that the percentage was still high (92.4%) with medium caries risk, indicating that these children are still at risk for developing

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Address for correspondence:
Dr. Armelia Sari Widyarman, Department of Microbiology, Faculty of Dentistry, Trisakti University, Jln. Kyai Tapa 260, Grogol Jakarta Barat, Jakarta 11440, Indonesia.
E-mail: armeliasari@trisakti.ac.id

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caries and need a different preventive treatment to reduce caries activity.[10]

One of the most common methods of reducing caries activities is to administer systemic or topical fluoride to prevent tooth cavity. A topical fluoride application such as a varnish can be used to prevent tooth caries and has been reported by several studies and confirmed by systematic data and meta-analyses.[11] Some studies have shown that fluoride varnish can reduce a patient's risk of developing tooth caries. Peterson showed that fluoride varnish was more effective than other forms of topical fluoridation in preventing tooth caries. Other studies have reported that fluoride is effective in preventing tooth caries in 75% of cases. Some studies also showed a positive effect of fluoride varnish on caries.[12]

Study reported that another treatment to reduce caries risk is topical casein phosphopeptides-amorphous calcium phosphate (CPP-ACP), which consists of fluoride to decrease the prevalence of *S. mutans* in children's saliva, and thus reduces the risk factors associated with tooth caries.[13] Fluoride varnishes release fluoride, whereas CPP-ACP acts as a reservoir for calcium phosphate. Saliva consists of calcium and phosphate ions, and hence the application of CPP-ACP can decrease enamel demineralization and increase the remineralization process.[14]

The effectiveness of fluoride varnish and CPP-ACP has been widely researched, and how these treatments interact with saliva's pH and reduce caries activity has become an important subject of research. Patel *et al*. proved that CPP-ACP application was much more effective than fluoride varnish in terms of reducing caries activity.[15]

Materials and Methods

Design and research subjects

This randomized clinical trial with the research subject population for this study comprised elementary students aged 8–9 years old being treated by the Faculty of Dentistry of Maranatha Christian University in Bandung, West Java, Indonesia. This research was approved by the Ethics Committee of the Faculty of Dentistry, Trisakti University (EC number 228/S2-Sp/KEPK/FKG/11/2018). The research subject number was obtained using multistage cluster random sampling. Saliva samples were obtained from 60 children before and after procedures. The children received topical fluoride once a week for 1 month. The sample was divided into two groups, each consisting of 30 children: Group 1 received fluoride varnish (Clinpro™ white varnish, manufactured by 3M ESPE, Morley Street Loughborough Leicestershire) containing 5% sodium fluoride), whereas Group 2 received CPP-ACP (MI varnish, manufactured by GC Corporation, Japan).

The examinations included saliva pH test (conducted using an Orion Star A221/Stara 2215 digital pH meter, manufactured by Thermo Scientific) and a caries activity test that involved measuring the quantity of *S. mutans* quantitative polymerase chain reaction (qPCR) present in the subjects' oral cavity using a 16srDNA primer genes of *S. mutans* (Macrogen USA 1330 Piccard Drive Rockville, MD, 20,850 United States).

Saliva sampling methods

Subjects were asked not to eat or drink 2 h before collecting unstimulated saliva. Saliva is collected between 9 a. m. and 11 a. m. Saliva sampling was carried out by the spitting method collected using a 5 ml sterile falcon tube as much as 5 ml. Salivary pH measurements were carried out using a digital pH meter (Orion Star A221/Stara 2215-Thermo Scientific) on a test tube containing salivary samples. Oral hygiene practice standards are explained to patients and reinforced at each visit. Saliva samples were collected and processed for the calculation of *S. mutans* quantity.

DNA extraction methods

DNA extraction methods were using the heat-shock method. Material preparation derived from saliva samples, phosphate buffer saline (PBS). Salivary samples were measured optical density$_{600}$ to determine their concentration, and then as much as 1 ml of saliva was centrifuged at 4500 ×g for 15 min. Pellet rinsed with 1 ml PBS, then vortexed again. Centrifugation returns at a speed of 10,000 ×g for 10 min. After that, the pellet is resuspended with 100 µL ddH_2O plus bead then vortexed for 5 min. After that, it is then heated in a 100°C water bath for 20 min, then incubated in ice for 10 min, then in the vortex. Centrifugation returns at a speed of 10,000 ×g for 2 min. Supernatant containing DNA is transferred to a new tube and stored at −20°C.

Quantitative polymerase chain reaction methods

The primary sequence was chosen to amplify specific parts of the *S. mutans* gene is reverse 5'-GGTTC(G/C) TTGTTACGACTT-3' and forward 5'-AGAGTTTGATC(A/C) TGGCTAC-3'. Amplification and quantification were carried out on a 96 well PCR plate. Each reaction contained 5 µL DNA template from disc extract, 10 µL Light Cycler 480 SYBR Green I Master (Roche diagnostics Gmbh, Germany), 1 µL each 10 µM forward and reverse primer. Double distilled water was added to adjust the reaction mixture to a final volume of 20 µL. Amplification and detection were carried out using Applied Biosystems Step One qPCR. Real-time PCR System with the following cycle profiles: Initial denaturation at 95°C for 10 min, followed by 40 amplification cycles at 95°C for 10 s, 65°C for 5 s, and 72°C for 11 s. Finally, the analysis of the melting curve is performed using the following cycling parameters: 95°C for 5 s, 65°C for 1 min and a slow increase in temperature until the final temperature of 97°C is reached. Each sample is run in

duplicate. Standard curves are constructed using threshold cycle computed tomography values of the corresponding qPCR and colony forming units (CFU)/mL. The amount of CFU/mL in the saliva sample is then quantified using this curve.

Statistical analysis

Salivary pH measurements were performed two times the measurement of salivary pH in each sample group (Group 1 and Group 2) before and after application, then the average values were taken. Caries activity measurement is done by measuring the quantity in two groups of applications obtained from saliva samples, each sample is measured before and after application with the qPCR measuring tool and converted into log CFU/ml units (using standard curves) then an average S quantity is assessed *S. mutans* before and after treatment**.** The data for comparing saliva pH and caries activity following treatment with fluoride varnish were analyzed using a *t*-test.

Results

Each subject's saliva pH was measured twice, before and after the course of treatment, to measure caries activity. The quantity of *S. mutans* was measured at the MiCORE Laboratory, used by the Faculty of Dentistry of Trisakti University. The caries activities test was performed by assessing each sample before and after treatment using qPCR; the data were then converted into log CFU/mL using a standard curve.[16]

The statistical analyses of the saliva pH and caries activity (quantity of *S. mutans*) measurements gathered from the sample population are presented in Table 1. For Group 1 (those treated with fluoride varnish), the data showed that there was a difference in saliva pH before and after treatment ($P < 0.05$). The data for Group 1 indicated that most of the subjects experienced a reduction of caries activity. Only five participants experienced a slight increase and one subject experienced a significant increase following treatment with fluoride varnish [Figure 1]. The data presented in Table 2 showed that there was a difference in the caries activity before and after treatment with fluoride varnish ($P < 0.05$).

The data for Group 2 (CPP-ACP) indicated that there was a difference in the subjects' saliva pH before and after treatment with CPP-ACP ($P < 0.05$) as shown on Table 3. The caries activity test results for both groups indicated a decrease of *S. mutans* in most subjects, although nine participants experienced an increase of *S. mutans* after treatment with CPP-ACP [Figure 2]. There was a difference in caries activity before and after treatment with CPP-ACP application ($P < 0.05$) [Table 4]. The statistical analyses of the data indicating the different effectiveness of fluoride varnish and CPP-ACP on saliva pH and caries activity were performed using a *t*-test [Tables 5 and 6].

Discussion

This study lasted for 1 month and compared the effects of fluoride varnish and CPP-ACP on saliva pH and caries activity. The subjects were aged 8–9 years old. The caries activity test was performed by measuring the quantity of *S. mutans* present in a patient's oral cavity using qPCR. The fluoride varnish used in this study was a single dose of Clinpro white varnish, which contained 5% sodium fluoride in addition to calcium fluoride and released fluoride ions.[17]

The results presented in Table 1 indicate that the participants' saliva pH increased after 1 month of treatment with fluoride varnish; this was confirmed by the statistical data. This treatment is effective in inhibiting the demineralization process, thereby slowing the progression of carious damage. The study results indicate that intensive treatment with a slow-releasing fluoride varnish is promising in increasing patients' saliva pH and slowing the demineralization of teeth.[16] Patil found that fluoride varnish releases a large amount of fluoride, thereby significantly enhancing the remineralization process and reducing caries.[18] The other treatment used in this research was CPP-ACP. Reynold stated that the anticariogenic property of CPP-ACP is substantially localized in the calcium and phosphate ions in plaque, thus creating a reservoir for calcium phosphate ions to

Table 1: Saliva pH before and after treatment with fluoride varnish

Fluoride varnish	*n*	Saliva pH, x±SD	*P*
Before treatment	30	6.9260±0.0378	0.000*
After treatment	30	7.0553±0.0344	

*Significance $P<0.05$. SD: Standard deviation

Table 2: Caries activity before and after treatment with fluoride varnish

Fluoride varnish	*n*	Caries activity (log CFU/mL), x±SD	*P*
Before treatment	30	7.518±2.165	0.000*
After treatment	30	4.329±2.637	

*Significance $P<0.05$. SD: Standard deviation; CFU: Colony-forming units

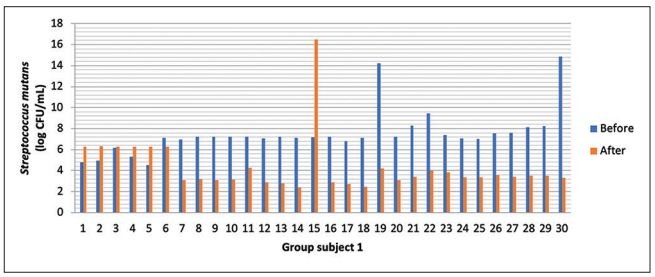

Figure 1: Caries activity (*Streptococcus mutans* number in the saliva) before and after treatment with fluoride varnish using quantitative polymerase chain reaction method

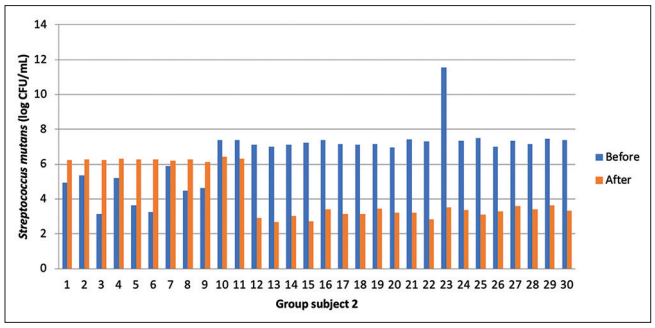

Figure 2: Caries activity (*Streptococcus mutans* number in the saliva) before and after treatment with casein phosphopeptides-amorphous calcium phosphate using the quantitative polymerase chain reaction method

dissolve on the tooth surface. In an acidic environment, CPP binds with ACP and releases calcium and phosphate ions. This process is ideal for preventing enamel demineralization because of the reverse relation between calcium level, plaque, and the phosphate concentration in the saliva.[19]

The statistical test results of this research indicate that the subjects' saliva pH increased following treatment with CPP-ACP [Table 3], indicating that treatment once a week for 1 month can increase saliva pH in children aged 8–9 years old. Saliva has a minimal potential for remineralization, as it acts only on the enamel surface. Mineral deposition on enamel lesions starts with the calcium and phosphate ions penetrating the enamel surface: CPP is an efficient means of remineralizing enamel because it removes acid from the enamel lesion. These results confirm the finding of Kargul *et al*. that 1 month of treatment with CPP-ACP decreased caries lesions by 77%.[20]

The different effects of fluoride varnish and CPP-ACP on the saliva pH of 8–9-year-old children in Sukajadi were statistically tested and found to be comparable [Table 5], indicating that both varnishes have advantages and that fluoride varnish remains the gold standard treatment for protecting teeth against white spot lesions and preventing demineralization. The statistical test indicated that treatment with fluoride varnish affected the quantity of *S. mutans* present in the subjects' oral cavities [Table 2], but not all of the subjects experienced the same degree of reduction [Figure 1].A few of the participants experienced an increase in *S. mutans* activity for a variety of reasons, including inappropriate tooth brushing and following a cariogenic diet.

According to Badjatia *et al*., the 1st month of treatment with fluoride varnish is significantly effective in reducing the quantity of bacteria in saliva. This effect can be sustained for the following 3 to 5 months of treatment, but after 6 months, the effect begins to deteriorate.[21]

In this study, although the application of fluorine varnish and CPP-ACP was only done once a week for 1 month, it resulted in a decrease in the quantity of *S. Mutans* in

Table 3: Saliva pH before and after treatment with casein phosphopeptides-amorphous calcium phosphate

CPP-ACP	*n*	Saliva pH, x±SD	*P*
Before treatment	30	6.9327±0.0239	0.000*
After treatment	30	7.0730±0.0349	

*Significance $P<0.05$. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate

Table 4: Caries activity before and after treatment with casein phosphopeptides-amorphous calcium phosphate

CPP-ACP	*n*	Caries activity (log CFU/mL), x±SD	*P*
Before treatment	30	6.568±1.663	0.000*
After treatment	30	4.331±1.515	

*Significance $P<0.05$. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate; CFU: Colony-forming units

Table 5: The difference between fluoride varnish and casein phosphopeptides-amorphous calcium phosphate in terms of effect on saliva pH

Group	*n*	Mean±SD	*P*
1 (treatment with fluoride varnish)	30	−0.12933±0.0397	0.256
2 (treatment with CPP-ACP)	30	−0.14033±0.0343	

*Significance $P<0.05$. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate

Table 6: The difference between fluoride varnish and casein phosphopeptides-amorphous calcium phosphate in terms of effect on caries activity

Group	*n*	Mean±SD, (log CFU/mL)	*P*
1 (treatment with fluoride varnish)	30	3.189±3.72	0.275
2 (treatment with CPP-ACP)	30	2.237±2.91	

*Significance $P<0.05$. CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate; SD: Standard deviation; CFU: Colony-forming units

number. Hashemi found that applying fluoride varnish once a week for 3 months significantly reduced caries incidence in the oral cavity.[22]

The other topical fluoride used in this research is CPP-ACP. This material is a topical fluoride that enhances the binding between fluoride and calcium ions in the oral cavity and increases the remineralization process to protect against early stage caries. Treatment with CPP-ACP produced the same results as fluoride varnish, namely the reduction of the quantity of *S. mutans*. This finding was confirmed by the statistical test [Table 4]. In this study, it was found that treatment with of CPP-ACP reduced the *S. mutans* in children aged 8–9 years. These results are similar to those of Jafari *et al*., who identified that CPP-ACP has an antibacterial effect. The right dosage of CPP-ACP is associated with a reduction in the quantity of bacteria.[23]

The results of the measurements and analysis indicate that there is no difference between fluoride varnish and CPP-ACP in terms of reducing the incidence of caries in children. Moreover, the data presented in Figures 1 and 2 indicate that there was a difference in quantity in the reduction of *S. mutans*. CPP-ACP and fluoride varnish each have their own benefits; a dentist, as a medical practitioner, can choose one treatment or use both because of their respective benefits, fluoride varnish because of its anticariogenic properties and CPP-ACP because of its ability to protect the structure of teeth. Either fluoride varnish or CPP-ACP can be administered regularly at elementary schools in Bandung to reduce the incidence of caries. Routine checking must be done to ensure that the desired preventive effect is being achieved.

Conclusion

The most effective treatment for increasing saliva pH and reducing caries activity can be achieved by using the varnish for 1 month. However, there is no difference between fluoride varnish and CPP-ACP with regard to altering saliva pH and reducing caries activity statistically. Future study is needed to explore this result and to analyze the mechanism of varnish to reduce the oral pathogens.

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Conflicts of interest

There are no conflicts of interest.

References

1. Welbury R, Duggal M. and Therese Hosey. Paediatric Dentistry. 4rd ed. Oxford: University Press; 2012. p. 56, 109-13.
2. Higham Susan. Caries Process and Prevention Strategies, Continuing education Course; 2011. Available from: http://www.dentalcare.com/en-US/dental-education/...ce369/ce369.aspx. [Last accessed on 2018 Aug 20].
3. Stookey GK, Chin JR, Kowolik JE. Dental Caries in the Child and Adolescent. Dentistry for the Child and Adolescent. 10th ed. St. Louis: Mosby Publications: 2015. p. 177-204.
4. Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans.* Microbiol Rev 1980;44:331-84.
5. Culp DJ, Robinson B, Parkkila S, Pan PW, Cash MN, Truong HN, *et al*. Oral Colonization by *Streptococcus mutans* and caries development is reduce upon detection of carbonic anhydrase VI expression in saliva. Biochim Biophys Acta 2011; 1812:1567-76.
6. Yang F, Zeng X, Ning K, Liu KL, Lo CC, Wang W, *et al.* Saliva microbiomes distinguish caries-active from healthy human populations. ISME J 2012;6:1-10.
7. Kardos T, dan Kieser J. Clinical Oral Biology. Vol. 11. 2nd ed. New Zealand: Unigraphics; 2000. p. 93-101.
8. Ahmadi-Motamayel F, Goodarzi MT, Hendi SS, Kasraei S, Moghimbeigi A. Total antioxidant capacity of saliva and dental caries. Med Oral Patol Oral Cir Bucal 2013;18:e553-6.
9. Denny PC. A saliva-based prognostic test for dental caries susceptibility. J Dent Hyg 2009;83:175-6.
10. Annual Report on Dental and Mouth Examination for Elementary School Children aged 6-7 years in Sukaja District. Bandung; 2016.
11. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2002;11(7):CD002279.
12. Mohammadi TM, Hajizamani A, Hajizamani HR, Abolghasemi B. Fluoride varnish effect on preventing dental caries in a sample of 3-6 years old children. J Int Oral Health 2015;7:30-5.
13. Fajriani dan Aini DH. Topical applications effect of casein phospho peptide-amorphous calcium phosphate and sodium fluoride on salivary Mutans Streptococci in children. Maj Ked Gigi 2014;47:110-4.
14. Ola B, dan Susan RA. Effect of application sequence of fluoride and CPP-ACP on remineralization of white spot lesions in primary teeth: An *In-vitro* study. Arch Oral Biol 2017:236-240.
15. Patel PM, Hugar SM, Math SM, BaDakar M, Gokhale S, Thakkar PC, *et al*. Comparison of the effect of fluoride Varnish, Chlorhexidine Varnish and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) Varnish on Salivary *Streptococcus mutans* Level: A six month clinical study. JCDR 2017;11:ZC53-9.
16. Seghatoleslami S, Ohlsson L, Hamberg K, Carlsson P, Ljunggren L, Ericson D. Quantitative detection of *Streptococcus mutans* from saliva using FTA TM elute cards and real-time polymerase chain reaction. AJMB 2013;3:148-52.
17. Patil SK, Fatangare M, Jadhav RG, Shinde GR, Pawar SS, Kathariya MD. Caries preventive effect of sodium fluoride varnish on deciduous dentition: A Clinical Trial J Contemp Dent Pract 2017;18:1190-3.
18. American Dental Association Council on Scientific Affairs. Professionally Applied Topical Fluoride: Evidence-Based Clinical Recommendations. J Am Dent Assoc 2006;137:1151-9.
19. Nidhi C, Anuj C. Enhanced remineralisation of tooth enamel using casein phospopeptide-amorphous calcium phosphate complex: A review. Int J Clin Pre Dent 2018;14:1-10.
20. Kargul B, Durmus B, Bekiroglu N. Effect of CPP-ACP on remineralisation of early caries lesions in primary teeth. OHDM 2017;16:1-4.
21. Badjatia S, Badjatia RG, Thanveer K, Krishnan AC. Effect of fluoride varnish on *Streptococcus mutans* count in saliva. IJCPD 2017;10:62-6.
22. Hashemi Z, Mohammadi TM, Poureslami H, Sharifi H. The effect of topical iodine and fluoride varnish combination in preventing early childhood caries: A pilot study. JDMT 2015;4:167-72.
23. Jafari K, Hekmatfar S Maryam F. *In vitro* comparison of antimicrobial activity of convetional fluoride varnish containing xylitol and casein phosphopeptide-amorphous calcium phosphate. JISPCD 2018;8:309-13.