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Research Article**Reduction of bacterial after irrigation with 6% NaOCl, and continued irrigation with 17%EDTA and 2%CHX during Endodontic Treatment of Chronic Apical Periodontitis**MARIA TANUMIHARDJA¹, REHATTA YONGKI¹, SITI WAHYUNI^{2a}, RASMIDAR SAMAD³, LATIEF MOODUTO⁴, YARIFUDDIN WAHID⁵¹Department of Endodontics, Dental Faculty, Hasanuddin University, South Sulawesi, Makassar, Indonesia 90245²Department of Parasitology, Medical Faculty, ¹Medical research Center, Hasanuddin University, South Sulawesi, Makassar, Indonesia 90245³Department of Dental Public Health, Dental Faculty, Hasanuddin University, South Sulawesi, Makassar, Indonesia 90245⁴Department of Endodontics, Dental Faculty, Airlangga University, East Java, Surabaya, Indonesia⁵Department of Pathology Anatomy, Immunology Division, Medical Faculty, Hasanuddin University, South Sulawesi, Makassar, Indonesia 90245

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1 ABSTRACT**Introduction:** Irrigants used during chemomechanical preparation of the root canal system play an essential role in the successful of endodontic treatment. Combination of irrigants have been recommended to optimize the elimination of bacteria.**Purpose:** This study was aimed to clinically evaluate the bacterial reduction following irrigation with 6% NaOCl, and continued irrigation with 17% EDTA and 2% CHX during endodontic treatment of chronic apical periodontitis.**Methods:** Samples from twenty four patients were recruited from Endodontic Department of Hasanuddin University Dental hospital. Exudates were collected from the root canals of upper anterior teeth before root canal preparation (S1 samples) and after manually-instrumented and irrigated using 6% NaOCl (S2 samples), continued irrigation with 17% EDTA and 2% CHX (S3 samples). All samples were incubated in blood agar and nutrient agar, evaluated for the bacterial load (CFU) using bacterial culture technique. Data were analyzed by Friedman test and Wilcoxon test.**Results:** All samples were positive for bacteria, and irrigations protocol either with 6% NaOCl, or 17% EDTA and 2% CHX, significantly reduced bacterial load respectively. Continued irrigation with 17% EDTA and 2% CHX showed significant bacterial reduction compared to irrigation with 6% NaOCl ($p < 0.05$).**Conclusion:** Continued irrigation after mechanical preparation in chronic apical periodontitis reduced the amount of bacteria, however it should be followed with intracanal medication to succeed endodontic treatment.**Keywords:** bacteria, single irrigation, continued irrigation, chronic apical periodontitis**INTRODUCTION**

Pulp and periapical pathoses were mainly caused by bacteria harbored in the root canal system. These bacteria are usually opportunistic pathogens that gain access to root canal and establish an infectious process. The infected root canal system acts as a reservoir of bacterial cells and its by products which evoke apical periodontitis. Essentially, endodontic infections are treated by chemomechanical preparation and an interappointment intracanal medication to eliminate the bacteria and control them (1-4). Chronic apical periodontitis (CAP) relates to the inflammation and destruction of periradicular

tissues, marked by the presence of an apical lesion (3). The presence of apical lesion and its size has been related to the bacterial virulence, number and types of bacterial in the root canal (5,6). For optimal treatment outcome of CAP, proper eradication or controlled of bacteria is of utmost important (2-4, 7-11). During mechanical instrumentation of the root canal system, the use of irrigating solutions with strong antimicrobial activity is recommended in order to further reduce bacterial numbers (2,4,8,9). Sodium hypochlorite (NaOCl) is mainly recommended as root canal irrigant in endodontic practice, and used in different concentrations



ranging from 1%-6% (14,2-14). It has a very good strength in dissolving the smear layer. However a layer of debris that occludes the dentinal tubules not be removed since NaOCl has lack of ability to remove the inorganic components of the smear layer. Therefore NaOCl should be combined with a chelating agent such as 17% ethylenediamine tetraacetic acid (EDTA) to completely remove both organic and inorganic components of smear layer, (7-14) which provide a fluid-tight seal of the root canal system (15). Interappointment intracanal medication has been advocated to achieve predictable disinfection in most cases, in particular, chronic apical periodontitis (16). However temporary materials used for coronal restoration can not adequately block the passage of bacteria into the disinfected canal that may impair the success of endodontic treatment (17). Chlorhexidine (CHX) has been studied and proposed as the final irrigant to provide the antimicrobial action during the short lifetime of a temporary coronal restoration, due to its ability to leave an antimicrobial residue on dentine (18-20).

For some clinicians, full strength concentration of sodium hypochlorite is preferred as a root canal irrigant (21) since the antibacterial effectiveness and tissue dissolution capacity are enhanced along with its concentration (12). To this date there is no consensus on the ideal concentration of sodium hypochlorite used (1,4,12). Besides sodium hypochlorite, some clinicians use 17% EDTA, and 2% CHX alternately, however limited data are available regarding their antibacterial effectiveness.

This study aimed to clinically evaluate the bacterial reduction after single irrigation with 6% NaOCl and continued irrigation with 17% EDTA and 2% CHX during endodontic treatment of teeth with chronic apical periodontitis. Culture microbiologic technique was used to ease the evaluation of bacterial reduction.

MATERIAL and METHODS

Patient selection

All the protocols and procedures were approved by the Ethics Committee of Medical Faculty (0892/H4.8.4.5.31/PP36-KOMETIK/2014), Hasanuddin University. Before the initiation of study, all patients were provided with informed consents.

Twenty four patients requiring primary root canal treatment were recruited from clinic of endodontic department, Oral and Dental Hospital, Hasanuddin University. The following inclusion criteria were non vital upper anterior teeth with straight root canal, rarefaction in apical area as shown by periapical radiography, no periodontal

seases, no systemic illness, no prescription of antibiotics or non-steroid anti-inflammatory in the last 3 month prior to the beginning of study.

Clinical procedures

The teeth were firstly isolated with rubber dam. External surfaces of the teeth and surrounding structure were disinfected with 30% hydrogen peroxide, followed by 2.5% NaOCl, then inactivated with 5% sodium thiosulfate to ensure bacteriologic sampling. Swab of external surfaces of the crown was done to guarantee their sterility by streaking it on blood agar plates. The preparation initiated using high-speed round diamond bur with sterile saline until pulp chamber was achieved. The access cavity was re-disinfected following the protocol described above, then root canal was explored. A sterile paper point (size 20; Dochem, China) was introduced into the full length of the canal, retained in position for 60 seconds for bacterial sampling (S1) before root canal preparation, and placed in a screw-cap container containing Stuart transport medium.

Preparation of root canal was done manually using hand K-files with back-and-forth alternated rotation motion (size 15/40 and 45/80; FKG Dentaire, Switzerland). Apex locator was used to establish the working length and confirmed by periapical radiography. Master apical files was set 3 sizes larger than initial apical files, and ranging of size 30-40. Root canal preparation was completed in one visit in all cases. During preparation, root canals were flushed with 3 mL of 6% sodium hypochlorite (local medical supplies) using a 27-gauge needle after each instrument size and second sampling (S2) were performed as the protocol mentioned above. Full strength concentration of 6% sodium hypochlorite was used as the diagnosis of the cases were long-term chronic apical periodontitis (>3 years). The next irrigants used were 3 mL of 17% EDTA, left in the canals for one minute, flushed with 2 mL sterile aquadest, and finally rinsed with 2% chlorhexidine digluconate (CHX), dried with sterile paper points. Final sampling (S3) was done with the same protocol. The samples were directly sent to the microbiology laboratory for microbial cultivation. Root canals were sterilized with calcium hydroxide paste (Ultradent, Utah, USA) using lentulo spiral fillers, packed with a cotton pellet at the level of canal entrance, confirmed with radiography, and the cavity was temporarily filled with Cavit-G (3M, ESPE, USA).

Bacterial loads: Samples were taken out from the container and put in the other container to be enriched with Brain-Heart Infusion Broth (BHIB), vortexed for 60 seconds to remove all bacteria and spread on BHIB. One mL of BHIB was added with



9 mL NaCl 0.9%, diluted and repeated in 3 series to achieve bacterial load of 10^3 CFU. Visual counts was done on appeared bacterial colonies.

Data Analysis

Data were not normally distributed (Shapiro-Wilk test). Therefore non-parametric test was used to quantitatively analyzed median of bacterial colony forming unit before chemomechanical preparation (S1) and after irrigation with 6% NaOCl (S2), and continued irrigation with 17% EDTA and 2% CHX (S3). Those values were then compared using Friedman and Wilcoxon test by SPSS v 21.0. The significance level was established at 5% ($p < 0.05$).

RESULTS

A total of 24 subjects were included in this study, consists of 7 males and 17 females, with age ranged from 16 to 38 years old.

The median number of bacterial colony forming units (CFU) in the initial/base line samples (S1) from the root canals were 24×10^3 (Min : 1- Max: 290). After irrigation with 6% NaOCl, the median number of bacterial colony forming units decreased to 11×10^3 CFU (Min: 0 - Max: 250), while continued irrigation with 17% EDTA and 2% CHX further decreased the median number of bacterial colony forming units to 2×10^3 CFU (Min : 0- Max: 97) (Table 1).

Irrigation with 6% NaOCl significantly reduced number of bacterial CFU compared to the initial samples, as well as continued irrigation with 17% EDTA and 2% CHX. Significant decrease of number of bacterial CFU was observed between continued irrigation with 17% EDTA and 2% CHX, and irrigation with 6% NaOCl alone (Table.2).

Table 1. Bacterial load before irrigation (S1), following irrigation with 6% NaOCl (S2), and continued irrigations with 17% EDTA, 2% CHX(S3)

Treatment	Bacterial load (10^3 CFU/ml)	p-value
	Median (Min-Max)	
S1	24 (1- 290)	0.000*
S2	11 (0-250)	
S3	2 (0-97)	

*Normality test, Shapiro-Wilk test: $p < 0.05$; data distribution not normal. *Friedman test: $p < 0.05$; significant

Medians bacterial load was significantly decreased before and after irrigation with 6% NaOCl, and continued irrigation with 17% EDTA and 2% CHX.

Table 2: Differences of bacterial colony forming unit (CFU/ml) before irrigation (S1), following irrigation with 6% NaOCl (S2), and continued irrigations with 17% EDTA, 2% CHX (S3)

Intervention	Comparison	Median Difference	p-value
S1	S2	13	0.001*
	S3	22	0.001*
	S3	9	0.029*

*Post Hoc test: Wilcoxon Sign Rank test; $p < 0.05$: significant

Medians bacterial load was significantly decreased for each of treatment compared to the initial samples.

Table 3: Incidence of positive bacterial detection before irrigation/initial (S1), following irrigation with 6% NaOCl (S2), and continued irrigations with 17% EDTA, 2% CHX (S3)

Initial (S1)	Sample	
	S2	S3
24/24 (100)*	22/24 (91.7)	18/24 (75)

*Number of positive samples/number of samples analyzed (percent)

Two samples (8.3%) were negative for bacteria after irrigation with 6% NaOCl, while 1 samples were negative for bacteria after continued irrigation with 17% EDTA and 2% CHX

DISCUSSION

Many studies have been conducted regarding the effectiveness of irrigation in eliminating the root canal bacteria either the types of irrigants used or

the techniques of irrigation to enhance those results. These show that irrigation is quite important as part of endodontic treatment (7-14). To dates, sodium hypochlorite is still an ideal irrigant with well documented antibacterial activity. However, no consensus exists of an ideal concentration of NaOCl used in endodontics (12,13). The preference of using a full-strength concentration of $> 5\%$ NaOCl has been reported



in 57% of 1102 American endodontists involving in a web-based survey. They proposed higher concentration of NaOCl has greater ability to kill the bacteria (21). This was relevant with the results reported by Clegg et al (2006) that 6% NaOCl was significantly effective against biofilm bacteria compared to 3% NaOCl (22), while dilution of NaOCl might reduce its antibacterial effectiveness and tissue dissolving capability (4). In contrary, tissue toxicity and caustic potential are also increased along with its concentration (23). However, other studies reported lower concentration of NaOCl showed similar antibacterial activities compared to 5.25% NaOCl (4, 12,13,16).

In the present study, irrigation with high strength of 6% NaOCl was chosen because mature biofilm is much more resistant to endodontic disinfecting agents (24), and 6% NaOCl was reported as an irrigant capable of disrupting the biofilm (22).

Following chemomechanical preparation which substantially reduced bacterial load (Tab.1). Using molecular microbiologic technique, Roqas and Siqueira (2015) reported 46% reduction of bacterial level after chemomechanical preparation and irrigation with 2.5% NaOCl (2). This might be explained by different technique used to elaborate the bacteria as molecular techniques have better sensitivity in detecting bacteria under laboratory artificial conditions ((2,16).

Several studies also reported no significant reduction of intracanal bacteria when high concentration of 5% NaOCl irrigant was used compared to lower concentration (8,16). This might be due to the inability of irrigants to physically contact the whole areas of root canal system during instrumentation. Sodium hypochlorite is unable to penetrate deeply into the canal irregularities and dentinal tubules due to its high surface tension (25). It is also suggested that needle insertion creates an air entrapment in apical region resulted in an air column and water column (air-solution system). These inhibit the solution exchange in the apical region which reduces its ability to effectively clean the root canal (26). Some other factors have been also shown to influence the efficacy of root canal irrigation, including irrigation volume, exposure time, method of delivery, pH, surfactant, apical preparation size and taper, distance of the irrigation needle to the apex, dimension of the irrigation needles, and root canal curvatures (12-14). In the present study, the volume of irrigant used was 3 mL, which might be inadequate to clean the canal although high concentration of irrigant was used.

Sodium hypochlorite has been known to have ability of removing inorganic components of the

smear layer. Further irrigation with 17% EDTA has been suggested to support the removal of inorganic component that occludes the dentine tubules. In addition, antibacterial effect of locally used disinfecting agents are also improved as biofilms were detached from root canal walls (12,27). Irrigation with 17% EDTA should be cautiously practised since it may inhibit the antibacterial activity and tissue dissolution of sodium hypochlorite by reducing free active chlorine (28,29). Therefore irrigation with 17% EDTA should be employed after complete chemomechanical preparation of the root canal system, flushed with aquadest, and followed by a final rinse of NaOCl (4,29).

Considering the fact that non vital root canal system is colonized by polymicrobial bacteria in nature, any measures is directed to optimize the elimination of those bacteria (2,5,6). Chlorhexidine (CHX) of 2% has been recommended as a final flush of root canal debridement of apical periodontitis since CHX adheres to root canal dentin that provides long-lasting antibacterial effect (11,15,30). In this study, irrigation with 2% CHX as a final irrigant following irrigation with 6% NaOCl and 17% EDTA, succeeded substantial reduction of bacterial load, and significant reduction was observed compared to irrigation with 6% NaOCl (Tab.2). Additional irrigation with CHX has been reported to provide antimicrobial action by preventing colonisation of microorganisms on to the dentinal walls (17,31). It was also observed that 21% of the samples were negative for bacteria by continued irrigation with 17% EDTA and 2% CHX compared to only 8.3% samples of using 6% NaOCl alone (Tab.3).

Although this study showed the efficacy of full strength concentration of sodium hypochlorite in reducing bacteria, clinicians should choose a lower concentration of sodium hypochlorite. Besides its high toxicity, high concentration of sodium hypochlorite produced high amount of extruded debris (9), that can generate inflammatory reaction in the periapical tissue (32).

The shortcoming of this study was that viable bacteria analyzed using culture anaerobic method could not detect bacteria below 10^3 compared to molecular techniques (33). In addition, conventional sampling procedures with paper points could not represent good sample from root canal system that might produce false negative results. However, besides its lack of sensitivity, this simple method may provide information about the effectiveness of disinfection means that account for the treatment outcome. Therefore any strategies should be determined to optimize eradication of those bacteria in further treatment.



CONCLUSION

Under the limitation of this study, it can be concluded that in addition to chemomechanical preparation with 6% NaOCl, continued irrigations with 17% EDTA and 2% CHX offers additional antimicrobial advantage, but should be followed by interappointment intracanal medication.

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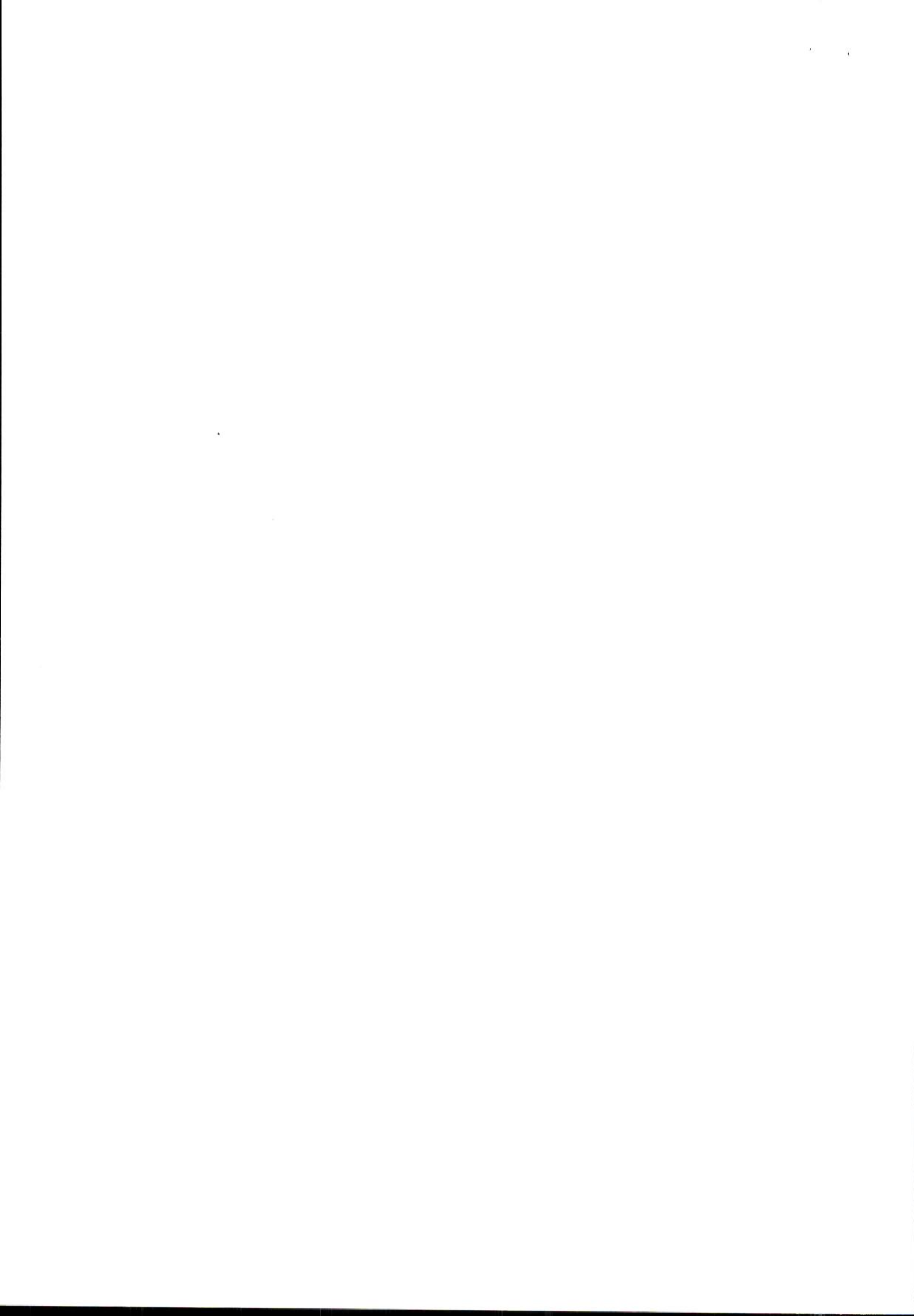
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