MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

Request of Revised

5 pesan

INSBIOMM Conference <insbiomm@gmail.com> 27 Desember 2019 pukul 09.31
Kepada: MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

Dear **Mr. Muhammad Luthfi,**

Thank you for submitting your manuscript to International Conference on Infectious Diseases, Biothreats, and Military Medicine **(INSBIOMM 2019)**

Your submission "ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND FREE CARIES" to International Conference on Infectious Diseases, Biothreats, and Military Medicine **(INSBIOMM 2019)** now needs to be revised.

Please revised this manuscript as peer the comment.

The revised itself is due : 2019-01-06

Best Regards
Scientific Committee

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Infectious Diseases, Biothreats, and Military Medicine
INSBIOMM 2019
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Phone/WhatsApp: +6281325267661

2 lampiran

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MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> 28 Desember 2019 pukul 18.46
Kepada: Institute Tropical Disease Universitas Airlangga <insbiomm@gmail.com>

Thank you, I will do that.

Pada tanggal Jum, 27 Des 2019 9:31 AM, INSBIOMM Conference <insbiomm@gmail.com> menulis:

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Phone/WhatsApp: +6281325267661

MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> 6 Januari 2020 pukul 12.01
Kepada: Institute Tropical Disease Universitas Airlangga <insbiomm@gmail.com>

selamat siang, berikut kami kirim kembali naskah dengan judul: **ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND CARIES FREE** yang telah kami revisi

terima kasih
luthfi

Pada tanggal Sab, 28 Des 2019 pukul 18.46 MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> menulis:

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17. Review R1 (revised- 2 januari 2020).doc
331K

INSBIOMM Conference <insbiomm@gmail.com> 7 Januari 2020 pukul 18.07
Kepada: MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

Dear **Author,**

Your revised manuscript is received. There will be the other reviewer that will be send.

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On Mon, Jan 6, 2020 at 12:01 PM MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> wrote:

selamat siang, berikut kami kirim kembali naskah dengan judul: **ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND CARIES FREE** yang telah kami revisi

terima kasih
luthfi

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MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> 9 Januari 2020 pukul 08.52
Kepada: INSBIOMM Conference <insbiomm@gmail.com>

Thanks a lot.

Pada tanggal Sel, 7 Jan 2020 6:08 PM, INSBIOMM Conference <insbiomm@gmail.com> menulis:

Dear **Author,**

Your revised manuscript is received. There will be the other reviewer that will be send.

Best Regards,
Scientific Committee

On Mon, Jan 6, 2020 at 12:01 PM MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> wrote:

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Inggris > Indonesia Terjemahkan pesan

Nonaktifkan untuk: Inggris

Dear **Author,**

The other reviewer of your submission "ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND FREE CARIES" to International Conference on Infectious Diseases, Biothreats, and Military Medicine (INSBIOMM 2019) now needs to be revised.

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Pesan Baru

MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

revisi okra (edited8-revised versi 6) dan gambar TIFF nya

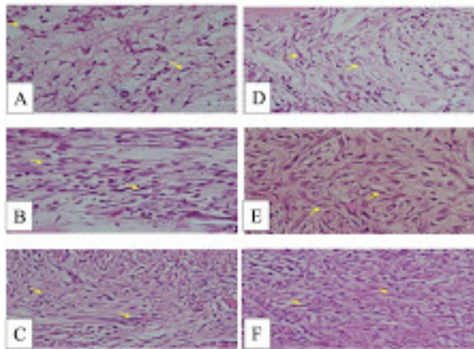
1 pesan

MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> 28 Februari 2020 pukul 10.21
Kepada: INSBIOMM Universitas Airlangga <insbiomm@itd.unair.ac.id>

Dear: Insbiomm comitte
berikut kami kirim naskah revisi okra dan gambar TIFF nya

Best regard
luthfi

2 lampiran



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ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND ~~FREE~~ CARIES FREE

ABSTRACT

Background: Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. Cariogenic bacteria are a significant risk factor for ECC associated with early colonization and high levels of cariogenic microbes (Streptococcus mutans (*S. mutans*). ~~lymphocyte~~ Lymphocyte T ($CD4^+$) cells known as helper T cells, are effector cells for mediated host immunity. N~~n~~aive T cells ($CD4^+$) must be activated to initiate effector function. ~~,~~ T~~t~~his activation occurs through interaction with professional antigen-presenting cells (pro-APC), especially dendritic cells that lead to intracellular pathways that regulate T cell receptor (TCR) more specifically against antigen in T cells.
Material and method: Lymphocyte cells from samples were collected from severe early childhood caries (S-ECC) and Free caries aged 5 to 6 years. The subjects were instructed to gargle 10 ml of sterile NaCl 1.5% solution for 30 seconds, and expectorate it into a sterile glass then analyzing T lymphocyte cell (CD4 +) expression using flow cytometry.
Results: lymphocyte T ($CD4^+$) cell expression at S-ECC (6.2525 ±, 64482) while in free caries (8.4138 ± 1.10397) with p-value (p = 0. 000).
Conclusion: of lymphocyte T ($CD4^+$) cells ~~c~~ E~~E~~xpression at S-ECC is lower than that occurring in free caries

Key words: *Severe Early Childhood Caries, adaptive immunity, lymphocyte T ($CD4^+$) cells Expression*

PENDAHULUAN

Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. ECC does not only affect the oral health of children, but also general body health (1). ECC not only involves pain in the oral cavity, orthodontic problems, and damage to the enamel, but can also cause problems with food intake, speech and increased risk for caries development in permanent teeth (2). ~~(Abanto et al., 2016).~~ Early loss of primary teeth often leads to orthodontic problems in adult life (3). ~~(Casamassimo et. al., 2009).~~

Early childhood caries (ECC) is the most common childhood chronic disease, with almost 1.8 billion new cases per year globally (4) ~~[Dye et al., 2012)~~ which occurs in about 37% of children aged 2-5 years in America States ~~(Dye et al., 2012)~~ and up to 73% of preschoolers who are socially economically disadvantaged in developing and industrialized countries (5). ECC is also highly prevalence in preschool children living in developing countries like Indonesia (6~~5b6~~) the prevalence of ECC in group of children aged 6 months - 3 years at Gunung Anyar Surabaya-Indonesaia was 30.8 % , while the prevalence was 29.2 % SECC. (7~~5c~~).
……

~~[Dye et al., 2015].~~ ECC was defined as the presence of ≥1 decay, loss (due to caries), or full tooth surface in primary teeth in children 71 months of age or younger. S-ECC occurs in

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Commented [dwi1]: What does this abbreviation means?
Commented [MOU2R1]: Severe Early Childhood Caries (S-ECC)
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children <3 years with ≥1 rot, missing (due to caries), or full tooth surface and in children aged 4-6 years with high caries score (86) ~~(Colak et al., 2013)~~. ECC and S-ECC remain serious problems that occur in school children in Xinjiang. Lower sociodemographic status (disadvantaged areas, low-educated mothers, low-income families, caregivers with cavities), risky dietary behavior (consumption of high frequency sweets, frequent meals before going to bed), oral hygiene behaviors that are at risk of ECC such as at what age start to brush teeth ~~risky oral hygiene behaviors (starting to brush teeth)~~ ~~at an age of age older),~~ and use of dental services (past dental visits, parents who have received oral health care instructions) are associated with an increased risk of ECC and S-ECC.

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Severe early childhood caries (S-ECC~~)~~ is an infectious disease that is a public health problem in the world, ~~–~~in spite of ongoing control efforts. The purpose of the host immune response during infection is to clear pathogens that attack with limited tissue damage. Both innate cells and adaptive T cells play a key role in clearing pathogens directly through the release of proinflammatory cytokines and the activity of cytotoxic T lymphocytes (CTL). In addition, helper (Th) T cells and regulatory Treg cells are required for antibodies secreted by plasma cells and immunomodulatory cytokines (eg, IL-10), respectively. In recent years, the role of the new set of Th cells, including follicular T cells namely Th17, Th22, in regulating anti-infective immunity, has become very important, because they play an important role in the development and outcome of disease (97). ~~(Liang et al., 2018).~~

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Cluster of differentiation 4 (CD4) coreceptor expressed in a subset of T cells, plays a role in differentiation, migration and cytokine expression (108). ~~(Zhen et. Al., 2014).~~ T cells involved in antigen recognition, CD4 stabilizes the ternary complex pMHC-TCR and CD4 recruits Lck kinase to phosphorylate ITAM and initiate intracellular signaling during activation of T cells induced by antigens (119). ~~(Artyomov et al., 2010).~~ CD4 was originally described as an adhesion molecule that enhances contact between T cells and precenting cell antigens. In their pillar work, Doyle and Strominger found direct correlations of other specific T cells involved in interactions (120). ~~(Doyle and Strominger, 1987).~~ CD4 binds MHCII molecules with very low 3D affinity ~~[see above;~~ ~~(Hoerter Jonsson et al., 2013~~2016)~~.~~(13). Based on the above background, the researchers wanted to analyze how the expression of T lymphocytes (CD4~~-~~+) cells in S-ECC and caries-free.

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MATERIAL AND METHODS

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This study was an analytic observational study, with cross-sectional analysis on two groups of sample; children with S-ECC and free caries children. All the procedures in this

study had been reviewed and approved by the Health Research Ethical Clearance Commission of Universitas Airlangga, Faculty of Dental Medicine, with certificate no 209/HRECC. FODM/IX/2017.

Lymphocyte Isolation

Lymphocyte cells from saliva obtained by instructing the subject to rinse with 10 ml of 1.5% sterile NaCl solution while rinsing, but not swallowed for 30 seconds, then expectorated in sterile glass. This procedure was repeated 4 times. The sample was then centrifuged at 450g for 15 minutes, at 40C. The centrifugation pellets were then mixed with 2 ml of RPMI medium, then the samples were vortexed (~~Gasparoto et al., 2011).~~14) The results of the filter in the form of cell suspension are then calculated using a hemocytometer.

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The same volume of cell suspension and 0.2% dye of trypan blue were mixed in the eppendorf tube and in doing vortex ~~divortex.~~ The same suspension aliquots (20 µl) were added to both chamber haemocytometers and observed under a microscope (10X objective). The mixture is withdrawn with capillary action. The cells are counted in an area of 16 squares which is equivalent to the number of cells x104 / ml. Only translucent cells are counted in the box. The number of cells per ml is calculated using the following formula:

Commented [dwi13]: Bahasa Indonesia?

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Cell / ml = average number of cells per primary square x 10^4 x dilution factor

Lymphocyte Culture and Cultivation

Lymphocyte cells ($3x10^5$cells/ml) were cultured in the tissue culture flask (Greiner) $75cm^2$ with complete culture medium (RPMI-1640, 10% fetal calf serum (FCS), and 1% penicillin/streptomycin) in 5% CO2 and atmosphere humidity 95% at 37°C for 24 hours. The cultures were checked daily to observe the changes in color, turbidity, density, and growth pattern using inverted light microscope (Nikon

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$CD4^+$ Expression Analysis

The expression of $CD4^+$ were observed by means of flow cytometry method adapted from (15~~Cherng et al (2008~~). Fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Peridinin chlorophyll protein (PerCP), PerCP-Cy5.5-conjugated

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monoclonal antibodies (mAbs) from Becton Dickinson (San Jose, CA, USA). The optimum concentration of mAbs were determined for each mAb by means of titration. Flow cytometry can both measure and analyze the physical characteristics of a particle such as cell since it can flow into the fluid stream through the light. The light scattered by the cell can be used to analyze changes in size, granularity, internal complexity, and relative fluorescence intensity (~~Zgonc dan Gruber 1998~~). Flow cytometry analysis is conducted to discover the immunomodulatory pattern of lymphocyte using conjugated monoclonal antibody.

Salivary lymphocytes were moved into FACS tube and washed with 4ml Dulbecco Phosphate Buffer Saline (DPBS~~), and~~) and centrifuged for 5 minutes at 2000rpm; the supernatant was subsequently removed. The pellet in DPBS were once again washed and centrifuged at 1800 rpm for 8 minutes. The cells were stained using yellow viability dye (1ml stain/1000µl DPBS) then vortexed and incubated at 4°C for 15 minutes. The cells were subsequently washed with 4ml DPBS and 1% FCS, centrifuged at 1800 rpm for 8 minutes and the supernatant was removed. The cells were stained with the exact required volume of mAbs, followed by vortexed and incubated in refrigerator for 20 minutes. After washed in cold DPBS and 1% FCS, cells were centrifuged for 8 minutes at 1800 rpm and the supernatant were removed. The cells were once again vortexed and 100µl of reagent A was added into the sample and cooled for 10 minutes. 50µl of mixture that had been fixated in reagent A was added into each ~~samples, and~~samples and covered with aluminum foil and stored in refrigerator until acquisition at LSR2 flow cytometry.

The stained lymphocytes were analyzed using flow cytometer (LSR 11 Sorvall RT7 Plus, Becton Dickinson, USA) with cell quest software (Becton Dickinson, USA). The results were analyzed using flow Jo 7.0 (USA) software. The expression of $CD8^+$ were analyzed using standard FACScan procedure with mAbs according to the producer protocol. The results are calculated and presented in mean.

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Statistical analysis

The acquired data was analyzed the normality and homogeny, then followed by T-test to find the difference between two groups, with the level of significance at 0.05.

RESULT

Data normality test using shapiro-Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t tes.

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Table 1. Mean and standard deviation of the expression of T lymphocytes (CD4 +) after 24-hour incubation were analyzed by flow cytometry test and statistical test t

No	Group	N	CD4+Expression	
			Mean (X)± SD	~~Standard deviasi (SD)~~p-value
1	S-ECC	8	6.2525 ± 0.64482	p= 0.0000~~0.64482~~
2	Free Caries	8	8.4138 ±1.10397	~~1.10397~~

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In table 1 shows that the mean expression of T lymphocytes (CD4 +) in S-ECC higher than caries free children.

~~Table 2. Test for normality of T lymphocyte (CD4 +) cell expression after incubation 24 hours analyzed by Flow Cytometry~~

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~~variabel~~	~~Kolmogorov-Smirnov[a]~~			~~Shapiro-Wilk~~		
	~~Statistic~~	~~df~~	~~Sig.~~	~~Statistic~~	~~df~~	~~Sig.~~
~~CD4+~~	~~.122~~	~~16~~	~~0.200~~	~~0.940~~	~~16~~	~~.345~~

~~Data normality test using shapiro-Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-~~

~~values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t test~~

~~Table 3. Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC group and free caries using the independent t test~~

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~~No~~	~~Group~~			
	~~S-ECC~~		~~Free Caries~~	
	~~Variabel~~	~~Mean Difference~~	~~Std. Error Difference~~	~~Sig. (2-tailed)~~
~~1~~	~~CD4~~	~~-2.16125~~	~~.45201~~	~~.000~~

Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC and free caries groups showed a p-value of 0,000, which is smaller than 0.05 (p <0.05), which means that there are significant differences between the S-ECC and free caries groups

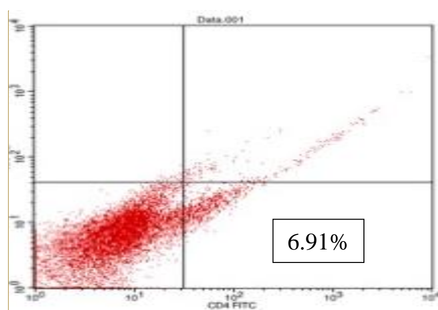

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva of~~the~~ ~~Free~~ Caries Free

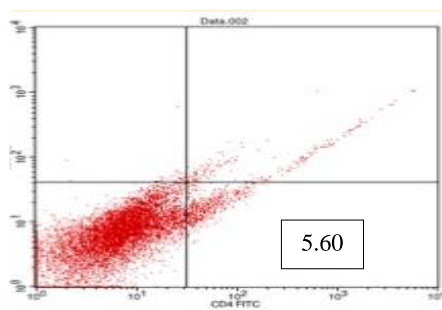

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of ~~Free~~ Caries Free

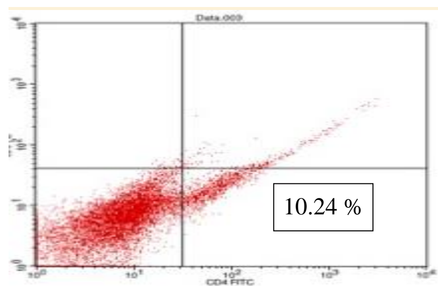

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

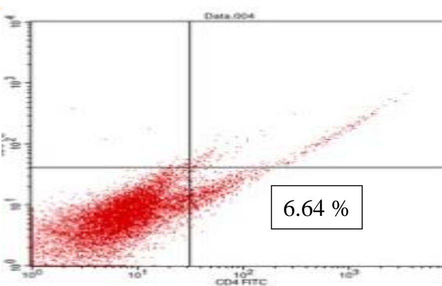

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

Commented [dwi20]: No explanation about this figure?

DISCUSSION

Steptococcus mutans (*S. mutans*) is the main bacterium that has a strong relationship with ECC while other oral bacteria in dental biofilms can be involved in the initiation and development of caries (16~~1~~). ~~(Hajishengallis et. Al., 2017).~~ Other bacteria associated with ECC are the Lactobacillus species which play an important role in the development of lesions (17~~2~~). ~~(Li and Tanner, 2015).~~ Actinomyces species, especially *Actinomyces gere~~n~~cseriae*, are also associated with caries initiation. in addition, some non-mutans streptococci that have acidogenic and aciduric~~akidurik~~ properties are also associated with dental caries. Epidemiological data indicate that in the pathogenesis of dental caries, *Candida albicans* also plays an active role ~~(~~(18~~3~~).~~Sukuraman and Pradeep, 2014).~~

T lymphocyte cells (CD4 +), known as helper T cells, are effector cells for cell-mediated immunity. T lymphocytes (CD4 +) are naive and must be activated to start effector functions, this activation occurs through interactions with professional "antigen-presenting cells (pro-APC) especially dendritic cells that lead to intracellular pathways that regulate T cell receptors (TCR) more specifically against antigens in T cells.

TCR and its co-receptors, such as CD4, form complexes with class 2 MHC receptors and antigens. CD4 + lymphocyte cells are then activated and produce cytokines to start the immune response of leukocyte cells or other immune cells of cell-mediated immunity and activate humoral immunity branches that depend on T cells, then CD4 + T cells recognize protein antigens and activate B cells to produce immunoglobulins in response to antigens (19,20~~4,15~~). ~~(Shen et. al., 2019, Bourne et. al., 2019).~~

The results of the study as shown in Table 1 show that the expression of T lymphocytes (CD4 +) cells in S-ECC is significantly lower than in free caries, this may cause the high *S. mutans* bacteria found in S-ECC saliva cannot be in

~~acquisition~~ a~~A~~cquisition by adaptive immunity because TCR and its co-receptors, such as CD4 which ~~have the ability to~~can form complexes with class 2 major receptor histocompatibility complex (MHC) receptors and antigens, cannot function optimally so that quantitatively the number of *S. mutans* which are bacteria that causes caries is higher ~~,~~ compared to caries-free children (21~~16~~). ~~(Lutfi et al., 2015).~~ Expression of T lymphocytes (CD4 +) in S-ECC causes the release of pro-inflammatory cytokines that function as ~~chemoatractants~~chemoattractant of neutrophil cells, because the movement of neutrophils

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toward the infection area is less than optimal, the movement of macrophages is also less than optimal towards the area of infection, giving *S. mutans* the opportunity to develop and do damage to the teeth

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In addition to the above, the low expression of CD4 + T lymphocyte cells in S-ECC ~~causes~~ results in slow B cells ~~to~~ forming antibodies ~~to slow~~. This happens because CD4 + T cells recognize antigens well and can activate B cells to produce antibodies in the form of immunoglobulins in response to *S. mutans* antigens.

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CONCLUSION

Low T lymphocyte ($CD4^+$) expression in S-ECC may be one of the causes of S-ECC

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Acknowledgment

The authors would like to thank Prof. Muhaimin Rifa'i, PhD.Med.Sc for the help in conducting this research.

Financial support and sponsorship

The authors would like to thank Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the grant funding provided for this research.

Conflicts of interest

The authors of this manuscript declare that they have no coflicts of interest, real or perceived, nancial or non- nancial in this article

REFERENCES

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1. Naidu R, Nunn J, Donnelly-Swift E. Oral health-related quality of life and early childhood caries among preschool children in Trinidad. BMC Oral Health [Internet]. 2016;16(1):1–9. Available from: http://dx.doi.org/10.1186/s12903-016-0324-7
2. Abanto J, Carvalho TS, Mendes FM, Wanderley MT, Bonecker M, Raggio DP. Impact of oral diseases and disorders on oral health-related quality of life of preschool children. Community Dent Oral Epidemiol. 2011 Apr;39(2):105–14.

3. Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E. Beyond the dmft: the human and economic cost of early childhood caries. J Am Dent Assoc. 2009 Jun;140(6):650–7.

4. Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected healthy people 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief. 2012;(104):1–8.

5. Dye BA, Hsu K-LC, Afful J. Prevalence and Measurement of Dental Caries in Young Children. Pediatr Dent. 2015;37(3):200–16.

65b. Amalia R. Indonesia. In: Folayan MO (ed). A Compendium on Oral Health of Children Around the World: Early Childhood Caries. New York, NY: Nova Biomedical; (2018). p. 155–65.

75c. *Rahel Wahjuni Sutjipto, Herawati Herawati, Satiti Kuntari, 2014. The prevalences of early childhood caries and severe early childhood caries in preschool children at Gunung Anyar Surabaya). j.djmkg.v47.i4.p186-189*

86. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med [Internet]. 2013 Jan;4(1):29–38. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23633832

97. Liang Y, Chen X, Chen J, Liu F. Differentiation and function of t cell subsets in infectious diseases. J Immunol Res. 2018;2018:2–4.

108. Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen SG. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. J Virol. 2014 Sep;88(17):9934–46.

119. Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. Proc Natl Acad Sci U S A. 2010;107(39):16916–21.

120. Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature [Internet]. 1987;330(6145):256–9. Available from: https://doi.org/10.1038/330256a0

13. Jonsson P, Jennifer H, Southcombe, Santos AM, Huo J et al.2016. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USA 113(20): 5682–5687

14. Luthfi M, Setijanto D, Rahardjo MB, Indrawati R, Rachmadi P, Ruth MS.(2019). Correlation between human neutrophil peptide 1–3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J;16:81-6

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15. Luthfi M, Oki AS, Indrawati R Latuamury NS (2019). Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries-Free in Surabaya. Malaysian Journal of Medicine and Health Sciences Vol.15 Supp 3:8-10

161. Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Mol Oral Microbiol [Internet]. 2017 Feb 1;32(1):24–34. Available from: https://doi.org/10.1111/omi.12152

172. Li Y, Tanner A. Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries. Pediatr Dent. 2015;37(3):226–44.

183. Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. Front Pediatr. 2017;5(July):1–7.

194. Shen H, Wu N, Nanayakkara G, Fu H, Yang Q, Yang WY, et al. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci (Landmark Ed. 2019 Jan;24:96–132.

2015. Bourne N, Perry CL, Banasik BN, Miller AL, White M, Pyles RB, et al. crossm Increased Frequency of Virus Shedding by Herpes Simplex. 2019;93(4):1–11.

2116. Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah Streptococcus mutans (S. mutans) dan Level Ekspresi Interlukin 8 (IL-8) pada Severe Early Childhood Caries. Maj Kedokt Gigi Indones. 2015;20(2):142.

Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proc Natl Acad Sci U S A.* (2010) 107:16916–21. doi: 10.1073/pnas.1010568107

Bourne N, Perry CL, Banasik BN, Miller AL, White M, Pyles RB, Schäfer H, Milligan GN. Increased Frequency of Virus Shedding by Herpes Simplex Virus 2-Infected Guinea Pigs in the Absence of CD4^{+} T Lymphocytes. J. Virol. 2019 Feb 15;93(4) [PMC free article] [PubMed]

Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013 Jan;4(1):29–38.

Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected Healthy People 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief. 2012 Aug;104:1–8.

Dye BA, Hsu KL, Afful J. Prevalence and measurement of dental caries in young children. Pediatr Dent. 2015 May-Jun;37(3):200–16.

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R.Naidu,J.Nunn,andE.Donnelly-Swift,“Oralhealth-related quality of life and early childhood caries among preschool children in Trinidad,” *BMC Oral Health*, vol. 16, no. 1, p. 128, 201

Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature.* (1987) 330:256–9. doi: 10.1038/330256a0

Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Molecular Oral Microbiology. 2017;**32**(1):24-34

J. Abanto, T. S. Carvalho, F. M. Mendes, M. T. Wanderley, M. Bonecker, and D. P. Raggio, “Impact of oral diseases and disorders on oral health-related quality of life of preschool children,” *Community Dentistry and Oral Epidemiology*, vol. 39, no. 2, pp. 105–114, 2011.

Jonsson P, Southcombe JH, Santos AM, Huo J, Fernandes RA, McColl J, et al. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. *Proc Natl Acad Sci U S A.* (2016) 113:5682–7. doi: 10.1073/pnas.~~1513918113 10.1084~~1513918113 10.1084/jem.20122528

Li Y, Tanner A. E ect of antimicrobial intervention on oral microbiota associated with early childhood caries. Pediatric Dentistry. 2015;37(3):226-244

Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah *Streptococcus mutans (S. mutans)* dan level ekspresi interlukin 8 (IL-8) pada severe early childhood caries. Maj Ked Gi Ind ~~2015;1:142~~2015; 1:142-8.

P. S. Casamassimo, S. ikkurissy, B. L. Edelstein, and E. Maiorini, “Beyond the dmft: the human and economic cost of early childhood caries,” *Journal of the American Dental Association*, vol. 140, no. 6, pp. 650–657, 2009.

Sukuraman A, Pradeep SA. Early childhood caries: Prevalence, risk factors, and preven- tion. Frontiers in Pediatrics. ~~2017;5:157~~2017; 5:157

Shen H, Wu N, Nanayakkara G, Fu H, Yang Q, Yang WY, Li A, Sun Y, Drummer Iv C, Johnson C, Shao Y, Wang L, Xu K, Hu W, Chan M, Tam V, Choi ET, Wang H, Yang X. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci (Landmark Ed). 2019 Jan 01;24:96-132.

Yan Li, Jibieke Wulaerhan, Yuan Liu, Ayinuer Abudureyimuand Jin Zhao. 2017. Prevalence of severe early childhood caries and associated socioeconomic and behavioral factors in Xinjiang, China: a cross-sectional study. BMC Oral Health 17:144 .1-10

Yuejin ~~Liang, Xiaojun~~Liang, Xiaojun Chen, Jinling Chen, Fengliang Liu, 2018. Differentiation and Function of T Cell Subsets in Infectious Diseases. Journal of Immunology Research Volume 2018. p1-2

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Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen S, et al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. *J Virol.* (2014) 88:9934–46.

10b. Jonsson P, Jennifer H, Southcombe, Santos AM, Huo J et al.2016. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USA 113(20): 5682–5687

10c. Luthfi M, Setijanto D, Rahardjo MB, Indrawati R, Rachmadi P, Ruth MS.(2019). Correlation between human neutrophil peptide 1–3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J;16:81-6

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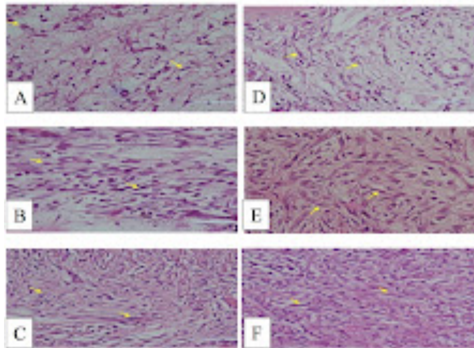

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INSBIOMM Conference <insbiomm@gmail.com> 27 Februari 2020 pukul 23.28
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Research Report

~~**EFFECT OF ADMINISTERING OKRA FRUIT (*Abelmoschus esculentus*) EXTRACT IN ACCELERATING WOUND HEALING THROUGH INCREASING FIBROBLAST CELL EXPRESSION**~~

Expression of fibroblast cells after extraction of wW~~w~~istar rat teeth after topical application of okra fruit (*A~~a~~belmoschus esculentus*) gel

Muhammad Luthfi[1]*, Wisnu Setyari Juliastuti[2], Yuniar Aliyah Risky[3]Elvina Hasna Wijayanti[43]Aisyah Ekasari Rachmawati[54], Nidya Pramesti Olifia Asyhari[65] ~~, Yuniar Aliyah Risky[6]~~

[1,2]Department of Oral Biology, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
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Acknowledgements : The author would like to thank all those who contributed to this research and to Research Centre Faculty of dental Medicine Universitas Airlangga

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Key words : tooth extraction, wound healing, fibroblasts, okra fruit.

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Contributions : The author contributes to starting to determine the topic of the problem, sampling, research and finally that all the authors approved the final version of the manuscript for publication.

Muhammad Luthfi: Study conception, study design, intelectual content, literature research, data acquisition, data analysis, manuscript review, guarantor

Wisnu Setyari Juliastuti: Study concept, clinical studies, experimental studies, data analysis, manuscript review

Yuniar Aliyah Risky,Elvina Hasna Wijayanti, Aisyah Ekasari Rachmawati, Nidya Pramesti Olifia Asyhari: data interpretation, Statistical analysis, manuscript preparation, manuscript editing

Conflict of Interests : The authors of this manuscript declare that they have no conflicts of interest in this article

Funding : The article is not funded or supported by any research grant

Clinical trials : in this study conducted pre clinical trials are in experimental animals

Conference presentation : International Conference on. Infectious Diseases, Biothreats, and Military Medicine (**INSBIOMM**)

Dedication : The author dedicates this research as a wound healing solution for diabetes mellitus

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Abstract

Background: Tooth extraction is a dental procedure for removing teeth from the alveolar bone socket. The tooth extraction process causes damage to hard tissue and soft tissue, so the body will respond physiologically to wound healing. ~~The process of wound healing after tooth extraction is a complex and dynamic process that aims to restore the network conditions~~

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~~as before. This process involves epithelial regeneration and the formation of connective tissue.~~ The wound healing process is divided into several phases, one of which is the proliferation phase of fibroblasts which is one of the important phases in the process of wound healing. Okra fruit contains saponins, tannins, flavonoids and alkaloids that have anti-inflammatory, antibacterial, antioxidant effects, and can stimulate angiogenesis so that it can accelerate the process of wound healing. **Objective**: to prove that the administration of okra fruit extract can accelerate the process of wound healing after extraction in the teeth of wistar rats through increased expression of fibroblast cells~~To determine that giving okra fruit extract (*Abelmoschus esculentus*) can increase fibroblast expression in wounds after extraction of wistar rat teeth~~. **Methods**: 18 wistar rats were divided into 2 groups; control group and treatment group. 30% okra fruit extract was given to the treatment group. The number of fibroblasts was calculated statistically using One Way ANOVA and Tukey HSD. **Results**: The results showed that the expression of control group fibroblast cells on day 3 (19.00 ± 2.0), day 5 (21.67 ± 2.08), day 7 (24.00 ± 2.00), whereas in the treatment group on day 3 (24.00 ± 1.00), day 5 (29.00± 2.00), day 7 (30.00 ± 1.53). Anova test between groups showed a significant difference with p-value 0.006~~, and the tukey HSD test showed a significant difference in the treatment group on day 3 compared to day 5 (0.018) after day 5 compared to day 7 (p = 0.006). There was a significant difference in group of day 3 compared to groups of day 5 and day 7, but there was no significant difference in group of day 5 compared to group of day 7.~~ **Conclusion**: 30% okra fruit extract ~~gel~~ can increase fibroblast expression in wound healing process after extraction of wistar rat teeth.

~~**Keywords**: Tooth extraction, wound healing, fibroblasts, okra fruit.~~

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Introduction

Tooth extraction is a dental procedure for removing teeth from the alveolar bone socket. The tooth extraction process causes damage to hard and soft tissue, and body will respond physiologically to wound healing.[1] The process of wound healing after tooth extraction is a complex and dynamic process that aims to restore the original condition of the network. This process involves epithelial regeneration and the formation of connective tissue, and depicts general principles that apply to wound healing in all tissues. The wound healing process is divided into several phases, namely the inflammatory phase, the proliferation phase, and the maturation/remodeling phase. The proliferation phase includes re-epithelialization, angiogenesis, granulation tissue formation, and collagen deposition starting on the fourth day for up to two weeks after injury.[2] Prevention on post-extraction wound healing complications is the most important factor, because when wound do not heal immediately, it will have an impact on public health and losses due to injuries can cause physical and psychological deficiencies, even death.[3]

Although there are several treatment options on the market for wound healing, many of them have high costs because they require long treatment.[4]Along with the times, the development of health pharmaceutical technology at this time has given more attention to natural ingredients, one of which can be used in wound healing. Natural ingredients are chosen because they are relatively safer when used compared to the use of chemical-based drugs.[5]

Okra fruit is a fruit that is widely available in Indonesia which is used by most people as a vegetable in daily food. One of the plants that can be used as an alternative treatment is

an okra fruit extract (*Abelmoschus esculentus*) because it has various medicinal properties such as antidiabetic, antioxidant, and antiplasmodial, antibacterial, anticancer, analgesic, antidiarrheal, and anti-inflammatory activities.[6] The active ingredients contained in okra fruit extract include saponins, tannins, flavonoids, and alkaloids.[7] The antioxidant content in okra fruit is quercetin which can protect the body from certain types of degenerative diseases. The saponin content functions as an antibacterial and can stimulate angiogenesis. ~~Research that has been done shows~~ Other studies have been reported that flavonoids have anti-inflammatory activity, moderator type III collagen sentiment, and also act as phospholipase inhibitors.[8] Flavonoids can also modulate oxidative burst in neutrophils which can cause a decrease in Reactive Oxygen Species (ROS) so that it can accelerate the process of wound healing.[9] Based on the above, much research from academics explores various new strategies to accelerate wound healing, including the use of plants and natural products. Based on the background above, we want to analyze the wound healing activity of okra (*Abelmoschus esculentus*) fruit extract in wistar rats.

Materials a~~A~~nd Methods

This research is an in vivo laboratory experimental study using a post-test only control group designs that have been tested ethical clearance in Airlangga University Faculty of Dental Medicine Health Research Ethical Clearance Commission Number: 155/HRECC. FODM/VII/2018.

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Samples

The samples used in this study were wistar rats obtained from the Biochemistry Laboratory Unit of the Medical Faculty of Airlangga University with the inclusion criteria were male wistar rats aged 2-3 months, wistar rats weighing 100-150 grams, while the exclusion criteria were male wistar rats who do not show active movements, decreased appetite~~do not want to eat~~, and diarrhea.

Samples that met the inclusion and exclusion criteria were divided into two groups, namely the treatment group given 30% okra fruit extract gel in as much as 0.1 ml given to the tooth extraction socket, while the control group was given a gel that did not contain okra fruit extract in as many as 0.1 ml given to the tooth extraction socket.

Okra (*Abelmoschus esculentus*) extract making procedure

Okra fruit extract derived from fresh okra fruit of Materia Medika Batu, Malang. ~~Fresh okra fruit is washed clean, then smoothed using a blender, and put into a jar with a tightly closed for 24 hours. Shake on a digital shaker at 50 rpm. The liquid extract is filtered with a cloth filter, then the extract collected in Erlenmeyer. The results of the liquid extract were evaporated with a rotary evaporator for 1 hour 30 minutes. The resulting extract is evaporated on the waterbath for 2 hours. After that the liquid extract that has been stored is stored.~~Fresh Okra fruit is then dried in a drying oven until a constant weight is reached. Dried fruit is then ground into powder. A total of 2 g of powder was extracted with 20 ml of 70% ethanol in a ratio of 1:10 (w / v) during the maceration period (24 hours) at room temperature. The mixture of solvent and soaked powder is filtered through filter paper then concentrated to 1

ml with a rotary evaporator and diluted with 5% dimethyl sulfoxide (DMSO) at a ratio of 1: 1 (v / v), and stored at -20° C until used more continued.~~.~~

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~~**Gel CMC Na 3% Making Procedure**~~

~~As the base material for the gel, Carboxy Methyl Cellulose Sodium (CMC Na 3%) was used. Making CMC Na 3% is by dissolving CMC Na 3% powder with warm water as much as 100 ml in the mortar. Giving the powder gradually and flat to the entire surface of the water so that it can be dispersed. Let stand and wait for 10-15 minutes to obtain a soft, transparent, gel-shaped mass. Then stir with the stamper until the gel becomes homogeneous and slowly add 40 ml of water so that the volume becomes 100 ml.~~

Gel okra fruit 30% extract making procedure

As the base material for the gel, Carboxy Methyl Cellulose Sodium (CMC Na 3%) was used. Making CMC Na 3% is by dissolving CMC Na 3% powder with warm water as much as 100 ml in the mortar. Giving the powder gradually and flat to the entire surface of the water so that it can be dispersed. Let stand and wait for 10-15 minutes to obtain a soft, transparent, gel-shaped mass. Then stir with the stamper until the gel becomes homogeneous and slowly add 40 ml of water so that the volume becomes 100 ml. Making 30% okra fruit extract gel was done by mixing 3 ml okra fruit extract and CMC Na 3% 7 ml.

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Preparation experimental animals

Male wistar rats aged 2-3 months weighing 100-150 mg were adapted in the same cage at a temperature of 25 ± 2 ° C, given pellet food and standard *ad libitium* distilled water for 7 days before the experiment started.[10] This procedure was done to reduce stress and obtain uniformity (homogeneity) of wistar rats conditions.

Experimental animal treatment

After adjusting for 7 days, 18 wistar rats were divided into 2 groups (control and treatment).

Wistar rats in each group were anesthetized by peritoneal injection using a 0.1 ml ketamine combination per rat. Waiting for 1-1.5 hours from the injection, then extracting the mandibular left incisor using a scalpel and needle holder. Make sure there are no leftover teeth left in the tooth socket. The tooth socket was then irrigated using saline solution.[11] In the control group after extraction it was left without being given an okra fruit extract gel but was given CMC Na 3% gel as much as 0.1 ml, while in the treatment group after extraction it was immediately given okra fruit extract on one apical third tooth socket as much as 0.1 ml.

Experimental animal euthanasia and mandibular extraction in the area of the tooth extraction socket

Wistar rats were sacrificed on the 3rd, 5th and 7th day after tooth extraction by lethal dose intraperitoneal injection of ketamine (minimum 4 times the anesthetic dose or about 0.4 ml / kgBB). Mandibular is taken from the temporo mandibular joint then wistar rats are buried according to the ethics of experimental animals. Mandibules in the incisor area were

cut vertically and then continued with preparation techniques in all groups with the paraffin method.[12]

HPA examination

samples obtained from wistar rat tooth socket i after extraction on days 3, 5 and 7 were analyzed histologically for expression of fibroblast cells by being fixed in 10% formalin at room temperature for at least 24 hours. After fixation, dehydrated in ethanol, cleaned with xylene, and carried out paraffin blocks (with a thickness of 6 mm) then deparafinated with xylene, prepared preparations

then stained with hematoxylin and eosin (HE) and then analyzed the expression of fibroblast cells under a microscope with a magnification of 400 X.

Statistical analysis

The data obtained is then carried out statistical analysis. To find out the data normally distributed, the Kolmogorov-smirnov test was carried out and then the homogeneity test was performed using the Levene Test. If the distribution is normal and the data is homogeneous

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then proceed with the One Way ANOVA test and if there are significant differences followed by the Tukey HSD test. If the data is normally distributed and the data is not homogeneous, the test performed is the Kruskall-Wallis test and if there are significant differences followed by the Mann-Whitney test.

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Results

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Based on research that has been done using 18 samples of Wistar rats (*Rattus norvegicus*) which were divided into 2 groups, namely the control group (K) and the treatment group (P). after treatment each group was sacrificed to count fibroblast cells and angiogenesis on days 3, 5, and 7. Examination of fibroblast cells was carried out under a light microscope at 400x magnification. Based on calculations, the results are as follows:For histological evaluation of fibroblast expression from sockets after extracting wistar rat teeth on days 3, 5 and 7, samples were then fixed in 10% formalin at room temperature for at least 24 hours. After fixation, dehydrated in graded ethanol, cleaned in xylene, and planted in paraffin. Embedded paraffin (in 6 mm thickness) then deparaffinized with xylene, dehydration aims to reduce ethanol concentration, then stained with hematoxylin and eosin (HE) and then analyzed fibroblast cell expression.

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Data Analysis

The data obtained is then carried out to statistical analysis. To find out the normally distributed data, the Kolmogorov-smirnov test was used. After the distribution test the homogeneity test was carried out using Levene Test. If the normal distribution and data are homogeneous then proceed with the One Way ANOVA test and if there are significant

differences followed by the Tukey HSD test. If the data is normally distributed and the data is not homogeneous then the test conducted is the Kruskall Wallis test and if there are significant differences followed by the Mann-Whitney test.[13]

RESULTS

Based on the research that has been done, using 18 samples of wistar rats (Rattus norvegicus) divided into 2 groups, namely the control group (K) and treatment (P). Each group was taken the preparation for HPA preparations in the former socket extraction of the lower jaw incisor wistar rats. In the control group, 30% okra fruit extract gel was not given in the tooth extraction socket, while the treatment group was given 30% okra fruit extract gel in the tooth extraction socket.

After extracting the mandibular left incisor, 3 rats from each group were sacrificed to calculate fibroblast cells and angiogenesis on day 3, 5, and 7. Examination of fibroblast cells was carried out under a light microscope with 400x magnification. Based on calculations, the results are as follows:

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Table 1. Mean and standard deviation of fibroblast cells after t-test.

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Groups	Samples	Mean ± standar deviation		
		Day 3	Day 5	Day 7
Control	3	19.00 ± 2.00	21.67 ± 2.08	24.00 ± 2.00
Treatment	3	24.00 ± 1.00	29.00± 2.00	30.00 ± 1.53

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The following is a graphical picture of the average number of fibroblast cells in the K and P groups on the 3rd, 5th and 7th day (Ffigure 1).

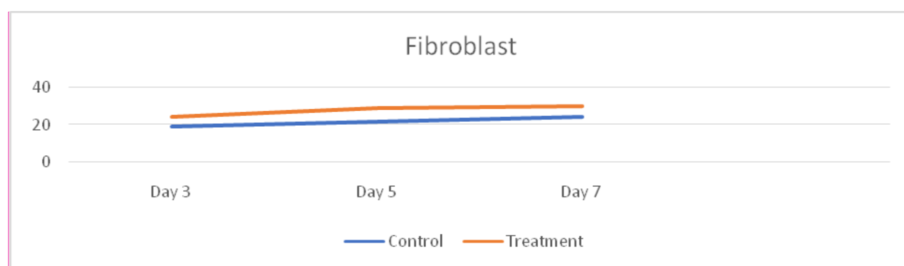

Figure 1. Graph of average number of fibroblast cells on days 3, 5, and 7. in the control group and the treatment of HE staining results were seen in a microscope with 400x magnification.

The calculation result of the number of fibroblasts in the tooth extraction sockets can be seen in Table 1 and Figure 1. On the 3rd day, the calculation of the number of fibroblast cells was obtained on average in the control group as many as 19, on the 5th day as many as 21.67, and on the 7th day as many as 24.

Table 2. The results of Anova test between the treatment group on days 3, 5, and 7.

ANOVA						
		Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Fibroblast	**Between groups**	66,889	2	33,444	13,682	0,006
	Within groups	14,667	6	2,444		
	Total	81,556	8			

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The ANOVA statistical test result on fibroblast cells and in Table 2 show the value of p = 0.006 (p <0.05) which means that there are significant differences in the number of fibroblast cells from each treatment group. To find out the significant differences in a group, the Tukey HSD test was carried out with α = 0.05.

Table 3. The results of the Tukey HSD test between the control group and the treatment group on days 3, 5, and 7.

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		Treatment groups		
		Treatment groups number of fibroblast cells		
	Day	**3**	**5**	**7**
Control Groups	**3**	0.018*		
	5		0.012*	
	7			0.012*

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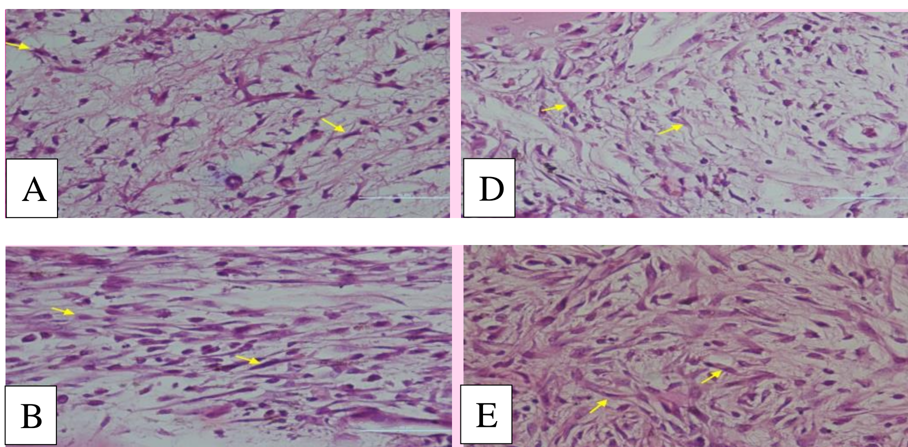
The results of the Tukey HSD test obtained and showed that there were significant differences between control groups and the treatment groups number of fibroblasts cells on day 3, 5, and 7.

Table 4. The results of the Tukey HSD test between the treatment groups on days 3, 5, and 7.

	Treatment groups number of fibroblast cells		
Day	**3**	**5**	**7**
3		p = 0.018*	p = 0.006*
5			p = 0.579
7			

The results of the Tukey HSD test showed that there were significant differences in the number of fibroblast cells in the treatment group between day 3 compared to day 5, but on day 5 compared to day 7 it did not show a significant difference.

The following is an overview of HPA expression of fibroblast cells in sockets after tooth extraction in the control group and treatment group as follows:



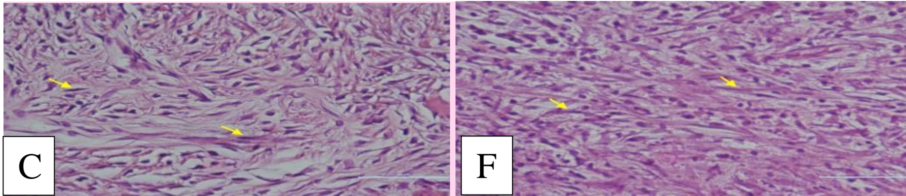


Figure 2. Description of HPA fibroblast cells in post tooth extraction sockets in group (A) Control of day 3, (B) Control of day 5, (C) Control of day 7, (D) Treatment of day 3, (E) Treatment of day 5, and (F) 7th day treatment. Arrow points to fibroblast cells with HE staining, 400x magnification.

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Discussion

On the histological examination of the results obtained, it was generally found that the mean number of fibroblast cells in the treatment group which was given a 30% okra fruit extract gel given after tooth extraction sockets seen on day 3, 5 and 7 experienced an increase compared to the control group. Based on statistical tests, there were significant differences in the treatment group on day 3 compared to day 5 and day 7, while on day 5 compared to day 7 there were differences in the number of fibroblast cells but did not show a significant difference. Whereas in the statistical test between the control group and the treatment group on the same day all showed significant differences.

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This is because many okra fruit extracts contain steroids, tannins, thiamin, amino acids, oxalic acid, and niacin. Besides okra fruit extract also contains important chemical compounds, namely flavonoids.[14] Okra (*Abelmoschus esculentus*) has an antioxidant and anti-inflammatory role.[15,16] Okra contains polyphenols and polysaccharides found in okra seeds and okra fruit skin, also contains flavonoids, iIsoquercetin, and quercetin-3-O-

gentiobiose contained in okra seeds and has an antioxidant effect.[17] Phytochemical analysis of herbal plants including okra, such as flavonoids, phenols and tannins. Tannins are phenolic compounds that are usually used in wound healing, while the astringent content serves to contract contractions and accelerate the process of epithelialization in the formation of granulation tissue and the remodeling phase.[18] whereas according to Talekar et al[19]., 2017 says that the extract content of the plant can accelerate the wound healing process by proliferation and mobilization of fibroblasts and keratiocytes, and promote angiogenesis at the wound site.

In this study on day 3 and 5 showed an increase of fibroblast cells because one of the roles of flavonoids contained in okra can reduce the release of prostaglandins and proinflammatory mediators by inhibiting the cyclooxygenase enzyme.[20] In addition, flavonoids can also reduce oxidative stress by regulating the activity of NF-kB, so that the increase in proinflamator cytokines can be reduced, okra fruit extract at 30% concentration is able to promote angiogenesis in post-extracted tooth socket of Wistar rats.[21]

The decrease causes inducible Nitric Oxide Synthase (iNOS) activity to be suppressed so that it can accelerate the wound healing process.[22] In a previous study by Pang et al. (2017) found that low-dose flavonoids alone were able to stimulate the expression of TGF-β growth factor which was able to increase TGF-β levels until the 7th day and in the wound healing process which is a stimulator of fibroblast cells.[23]

Conclusion

Giving an okra 30% fruit extract gel in the tooth socket after extraction can increase fibroblast cell proliferation.

References

1. Ngangi RS, Mariati NW, Hutagalung BSP. 2013. Gambaran pencabutan gigi di balai pengobatan rumah sakit gigi dan mulut Universitas Sam Ratulangi tahun 2012. Pp. 81-87.
2. Gonzales AC, Andrade ZA, Costa TF, Medrado AR. ~~2016.~~ Wound healing – a literature review. An Bras Dermatol 2016~~.~~; 91(5): 614-20.
3. Bayat A~~.~~, Mcgrouther D~~.~~ A~~.~~, and Ferguson M~~.~~W~~.~~J.~~2013.~~ Skin scarring.~~,”~~ *British Medical Journal* 2013~~,~~; ~~vol.~~ 326~~,~~: ~~pp.~~ 88–92.
4. Ferreira E, Lucas, R.. Rossi, L. A and Andrade D. ~~2003.~~ Curativo do paciente queimado: uma revisão de literatura. ~~,”~~ *Revista da Escola de Enfermagem da USP* 2003;~~, vol.~~ 37(~~, no.~~ 1)~~:~~~~, pp.~~ 44–51.
5. Auliyah, NS. 2015. Pengaruh Ekstrak Teripang Pasir terhadap Jumlah Sel Fibroblas pada Soket pasca Pencabutan Gigi Cavia cobaya. *Oral surg. Dent. J.* vol. 4, no. 2 2015-01. h: 1.
6. Nesa Luthfun, Islam Monirul, Alam Badrul, Munira Shirajum, Mollika Shabnam, Naher Mazmun, Islam Rafiqul. 2014. Analgesic, Anti-inflammatory and CNS Depressant Activities of the Methanolic Extract of Abelmoschus esculentus Linn. Seed in Mice. British Journal of Pharmaceutical Research, 4(7): 849-860.
7. Bello HS~~.~~, Mustapha A~~.~~, Isa MA~~.~~, and Rahila TC. ~~2015.~~ The invitro antibacterial activity of Okra (Abelmoschus esculentus) against some selected bacteria from Maiduguri, North Eastern Nigeria. International Journal of Biosciences and Nanosciences 2015;~~Vol.~~2(4): ~~pp.~~84-~~8~~8.

8. Kumar~~,~~ D~~.~~, Tony~~,~~ D~~.~~, Kumar~~,~~ A~~.~~, ~~Kumar, K., Rao, D., and Nadendla, Ramarao~~et al. ~~(2013).~~ A review on: Abelmoschus esculentus (Okra). Int. Res J Pharm. App Sci 2013;~~.;~~ 3~~(4)~~:129-~~1~~32.
9. Soemarie, Yulistia B. ~~2016.~~ Uji aktivitas antiinflamasi kuersetin kulit bawang merah (Allium cepa L.) pada mencit putih jantan (Mus musculus). Jurnal Ilmiah Ibnu Sina 2016~~,~~;~~ ~~1~~(2)~~;~~,~~ 163-~~1~~72.
10. Talubmook C, Nopparat Buddhakala. ~~2013.~~ Bioactivities of extracts from T~~t~~inospora crispa stems, A~~a~~nnona squamosa leaves, M~~m~~usa sapientum flowers, and P~~p~~iper sarmentosum leaves in diabetic rats. Journal of Advancements in Research & Technology 2013~~,~~;~~ ~~2~~(6)~~:14.
11. Gunawan~~,~~ F~~.~~, Sularsih, dan Soemartono. ~~2015.~~ Perbedaan kitosan berat molekul rendah dan tinggi terhadap jumlah sel limfosit pada proses penyembuhan luka pencabutan gigi. Denta Jurnal Kedokteran Gigi 2015;~~ Vol.~~ 9~~(1)~~: 114-~~1~~22.
12. Sudiana, IK. ~~2004.~~ Pewarnaan apoptosis. In: Teknologi Ilmu Jaringan dan Imunohistokimia. Jakarta: Sagung Seto; 2004.~~.~~ pp.9-47.
13. Sugiyono~~. 2008.~~. Metode penelitian pedidikan pendekatan kuantitatif, kualitatif, dan R&D. Bandung: Alfabeta; 2008.~~.~~
14. Roy~~,~~ A~~.~~, Shrivastava~~,~~ S~~.~~L~~.~~, ~~&~~ Mandal~~,~~ S~~.~~M. ~~2014.~~ Functional properties of Okra Abelmoschus esculentus L. (Moench): traditional claims and scientific evidences. Plant Science Today~~,~~ 2014.;~~ ~~1~~(3)~~;~~,~~ 121-~~1~~30.
15. Xia~~,~~ F~~.~~, Zhong~~,~~ Y~~.~~, Li~~,~~ M~~.~~, ~~Chang, Q., Liao, Y., Liu, X., Pan, R~~et al. ~~2015.~~ Antioxidant and anti-fatigue constituents of okra. Nutrients 2015:~~; p.~~ 8846-~~884~~7.
16. Solomon~~,~~ S~~.~~, Muruganantham~~,~~ N~~.~~, Senthamilselvi~~,~~ M~~.~~M. Anti-oxidant and anti-inflammatory activity of A~~a~~belmoschus esculentus (flowers). Indo American Journal Of Pharmaceutical Sciences;3(6):p. 605.

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17. Athira, C dan Jayaraman, J. ~~2018.~~ A review on: a pharmacological properties of A~~a~~belmoschus esculentus. World Journal of Pharmaceutical Research 2018-;7~~(12)~~: ~~p.~~ 165~~-, 1~~67.

18. Bin Li, Wang James HC. ~~2011.~~ Fibroblasts and myofibroblasts in wound healing: force generation and measurement. J Tissue Viab 2011;20~~(4)~~:108-20.

~~17.~~19. Talekar YP~~.~~, Apte KG~~.,~~ Paygude SV~~.,~~ ~~Tondare PR., Parab PB.,~~et al. ~~2017.~~ Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. Journal of Ayurveda and Integrative Medicine 2017;8:~~;~~73-81

20. Rathee Permender., Chaudhary Hema., Rathee Sushila., Rathee Dharmender, Kumar Vikash., Kohli Kanchan. ~~2009.~~Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflammation & Allergy - Drug Target 2009;8:229-~~2~~35.

~~18.~~ Luthfi M, Juliastuti WM, Risky YA. Angiogenesis of Extracted Tooth Wound on Wistar Rats After Application of Okra (Abelmoschus esculentus) Gel Extract. Pesqui Bras Odontopediatria Clín Integr. 2020; 20:e5087. https://doi.org/10.1590/pboci.2020.037

21.

~~19.~~ Qing, C. ~~2017.~~ The molecular biology in wound healing & non-healing wound. Chinese Journal of Traumatology 2017;20:~~p.~~ 189.

22.

23. Pang X, Bao D, Wang J, Liu H. ~~2017.~~ Protective effect of quercetin against oxidative stress-induced cytotoxicity in rat pheochromocytoma (PC-12) cells. *Molecules* 2017;~~s.~~ 22:~~,~~ 1122.

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Figure 1. Graph of average number of fibroblast cells on days 3, 5, and 7 in the control group and the treatment of HE staining results were seen in a microscope with 400x magnification.

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Figure 2. Description of HPA fibroblast cells in post tooth extraction sockets in group (A) Control of day 3, (B) Control of day 5, (C) Control of day 7, (D) Treatment of day 3, (E) Treatment of day 5, and (F) 7th day treatment. Arrow points to fibroblast cells with HE staining, 400x magnification.

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Analysis of lymphocyte t (cd4 +) cell expression on severe early childhood caries and free caries

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Acknowledgements :

The authors would like to thank Prof. Muhaimin Rifa'i, PhD.Med.Sc for the help in conducting this research.

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~~**Title**~~

Key words: Severe early childhood caries, adaptive immunity, lymphocyte T ($CD4^+$) cells expression

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Conflicts of interest

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Funding:

The authors would like to thank Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research,

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Technology and Higher Education of the Republic of Indonesia for the grant funding provided for this research.

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~~ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND FREE CARIES~~ FREE

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ABSTRACT

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~~**Background:**~~ Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. Cariogenic bacteria are a significant risk factor for ECC

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associated with early colonization and high levels of cariogenic microbes (Streptococcus mutans (*S. mutans*). ~~lymphocyte~~ Lymphocyte T ($CD4^{+}$) cells known as helper T cells, are effector cells for mediated host immunity. N~~n~~aive T cells ($CD4^{+}$) must be activated to initiate effector function.~~,~~ T~~t~~his activation occurs through interaction with professional antigen-presenting cells (pro-APC), especially dendritic cells that lead to intracellular pathways that regulate T cell receptor (TCR) more specifically against antigen in T cells.

~~**Material and method:**~~ Lymphocyte cells from samples were collected from severe early childhood caries (S-ECC) and Free caries aged 5 to 6 years. The subjects were instructed to gargle 10 ml of sterile NaCl 1.5% solution for 30 seconds, and expectorate it into a sterile glass then analyzing T lymphocyte cell (CD4 +) expression using flow cytometry.

~~**Results:**~~ L~~l~~ymphocyte T ($CD4^{+}$) cell expression at S-ECC (6.2525 ±, 64482) while in free caries (8.4138 ± 1.10397) with p-value (p = 0. 000). Conclusion

~~**Conclusion:**~~ of lymphocyte T ($CD4^{+}$) cells e~~E~~xpression at S-ECC is lower than that occurring in free caries.~~s~~

~~**Key words**: *Severe Early Childhood Caries, adaptive immunity, lymphocyte T ($CD4^{+}$) cells Expression*~~

~~PENDAHULUA~~INTRODUCTION~~N~~

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Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. ECC does not only affect the oral health of children, but also general body health.[(1)] ECC not only involves pain in the oral cavity, orthodontic problems, and damage to the enamel, but can also cause problems with food intake, speech and increased risk for caries development in permanent teeth.[(2)] (Abanto et al., 2016). Early loss of primary teeth often leads to orthodontic problems in adult life .[(3)] (Casamassimo et. al., 2009). Early childhood caries (ECC) is the most common childhood chronic disease, with almost 1.8 billion new cases per year globally [(4)] [Dye et al., 2012) which occurs in about 37% of children aged 2-5 years in America States (Dye et al., 2012) and up to 73% of preschoolers who are socially economically disadvantaged in developing and industrialized countries.[(5)] ECC is also highly prevalence in preschool children living in developing countries like Indonesia [(6] 5b6) the prevalence of ECC in group of children aged 6 months - 3 years at Gunung Anyar Surabaya-Indonesaia was 30.8 % , while the prevalence was 29.2 % SECC. [(7] 5c).

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[Dye et al., 2015]. ECC was defined as the presence of ≥1 decay, loss (due to caries), or full tooth surface in primary teeth in children 71 months of age or younger. S-ECC occurs in children <3 years with ≥1 rot, missing (due to caries), or full tooth surface and in children aged 4-6 years with high caries score.[(86)] (Colak et al., 2013). ECC and S-ECC remain serious problems that occur in school children in Xinjiang. Lower sociodemographic status (disadvantaged areas, low-educated mothers, low-income families, caregivers with cavities), risky dietary behavior (consumption of high frequency sweets, frequent meals before going to bed), oral hygiene behaviors that are at risk of ECC such as at what age start to brush teeth risky oral hygiene behaviors (starting to brush teeth) at an age of age older), aand use of

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dental services (past dental visits, parents who have received oral health care instructions) are associated with an increased risk of ECC and S-ECC.

Severe early childhood caries (S-ECC~~)~~ is an infectious disease that is a public health problem in the world, ~~-~~in spite of ongoing control efforts. The purpose of the host immune response during infection is to clear pathogens that attack with limited tissue damage. Both innate cells and adaptive T cells play a key role in clearing pathogens directly through the release of proinflammatory cytokines and the activity of cytotoxic T lymphocytes (CTL). In addition, helper (Th) T cells and regulatory Treg cells are required for antibodies secreted by plasma cells and immunomodulatory cytokines (eg, IL-10), respectively. In recent years, the role of the new set of Th cells, including follicular T cells namely Th17, Th22, in regulating anti-infective immunity, has become very important, because they play an important role in the development and outcome of disease.[(97)] ~~. (Liang et al., 2018).~~

Cluster of differentiation 4 (CD4) coreceptor expressed in a subset of T cells, plays a role in differentiation, migration and cytokine expression.[(108)] ~~. (Zhen et. Al., 2014).~~ T cells involved in antigen recognition, CD4 stabilizes the ternary complex pMHC-TCR and CD4 recruits Lck kinase to phosphorylate ITAM and initiate intracellular signaling during activation of T cells induced by antigens.[(119)] ~~. (Artyomov et al., 2010).~~ CD4 was originally described as an adhesion molecule that enhances contact between T cells and precenting cell antigens. In their pillar work, Doyle and Strominger found direct correlations of other specific T cells involved in interactions[(120)] ~~. (Doyle and Strominger, 1987).~~ CD4 binds MHCII molecules with very low 3D affinity. ~~[see above; (Hoerter Jonsson et al., 20132016).~~ [(13)]. Based on the above background, the researchers wanted to analyze how the expression of T lymphocytes (CD4~~-~~+) cells in S-ECC and caries-free.

MATERIAL AND METHODS

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This study was an analytic observational study, with cross-sectional analysis on two groups of sample; children with S-ECC and free caries children. All the procedures in this study had been reviewed and approved by the Health Research Ethical Clearance Commission of Universitas Airlangga, Faculty of Dental Medicine, with certificate no 209/HRECC. FODM/IX/2017.

Lymphocyte Isolation

Lymphocyte cells from saliva obtained by instructing the subject to rinse with 10 ml of 1.5% sterile NaCl solution while rinsing, but not swallowed for 30 seconds, then expectorated in sterile glass. This procedure was repeated 4 times. The sample was then centrifuged at 450g for 15 minutes, at 40C. The centrifugation pellets were then mixed with 2 ml of RPMI medium, then the samples were vortexed. ~~(Gasparoto et al., 2011).14~~ The results of the filter in the form of cell suspension are then calculated using a hemocytometer.

The same volume of cell suspension and 0.2% dye of trypan blue were mixed in the eppendorf tube and in doing vortex ~~divortex.~~ The same suspension aliquots (20 µl) were added to both chamber haemocytometers and observed under a microscope (10X objective). The mixture is withdrawn with capillary action. The cells are counted in an area of 16 squares which is equivalent to the number of cells x104 / ml. Only translucent cells are counted in the box. The number of cells per ml is calculated using the following formula:

Cell / ml = average number of cells per primary square x 10^4 x dilution factor

Lymphocyte Culture and Cultivation

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Lymphocyte cells (3x10^{5}cells/ml) were cultured in the tissue culture flask (Greiner) 75cm^2 with complete culture medium (RPMI-1640, 10% fetal calf serum (FCS), and 1% penicillin/streptomycin) in 5% CO_2 and atmosphere humidity 95% at 37°C for 24 hours. The cultures were checked daily to observe the changes in color, turbidity, density, and growth pattern using inverted light microscope (Nikon

CD4⁺ Expression Analysis

The expression of CD4$^+$ were observed by means of flow cytometry method adapted from. [15] ~~Cherng et al (2008).~~ Fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Peridinin chlorophyll protein (PerCP), PerCP-Cy5.5-conjugated monoclonal antibodies (mAbs) from Becton Dickinson (San Jose, CA, USA). The optimum concentration of mAbs were determined for each mAb by means of titration. Flow cytometry can both measure and analyze the physical characteristics of a particle such as cell since it can flow into the fluid stream through the light. The light scattered by the cell can be used to analyze changes in size, granularity, internal complexity, and relative fluorescence intensity ~~(Zgonc dan Gruber 1998)~~. Flow cytometry analysis is conducted to discover the immunomodulatory pattern of lymphocyte using conjugated monoclonal antibody.

Salivary lymphocytes were moved into FACS tube and washed with 4ml Dulbecco Phosphate Buffer Saline (DPBS~~), and~~) and centrifuged for 5 minutes at 2000rpm; the supernatant was subsequently removed. The pellet in DPBS were once again washed and centrifuged at 1800 rpm for 8 minutes. The cells were stained using yellow viability dye (1ml stain/1000µl DPBS) then vortexed and incubated at 4°C for 15 minutes. The cells were subsequently washed with 4ml DPBS and 1% FCS, centrifuged at 1800 rpm for 8 minutes and the supernatant was removed. The cells were stained with the exact required volume of mAbs, followed by vortexed and incubated in refrigerator for 20 minutes. After washed in cold

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DPBS and 1% FCS, cells were centrifuged for 8 minutes at 1800 rpm and the supernatant were removed. The cells were once again vortexed and 100µl of reagent A was added into the sample and cooled for 10 minutes. 50µl of mixture that had been fixated in reagent A was added into each ~~samples, and~~samples and covered with aluminum foil and stored in refrigerator until acquisition at LSR2 flow cytometry.

The stained lymphocytes were analyzed using flow cytometer (LSR 11 Sorvall RT7 Plus, Becton Dickinson, USA) with cell quest software (Becton Dickinson, USA). The results were analyzed using flow Jo 7.0 (USA) software. The expression of $CD8^+$ were analyzed using standard FACScan procedure with mAbs according to the producer protocol. The results are calculated and presented in mean.

Statistical analysis

The acquired data was analyzed the normality and homogeny, then followed by T-test to find the difference between two groups, with the level of significance at 0.05.

RESULT

Data normality test using shapiro-Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t tes.

Table 1. Mean and standard deviation of the expression of T lymphocytes (CD4 +) after 24 hour incubation were analyzed by flow cytometry test and statistical test t

No	Group	N	CD4+ eExpression	
			Mean (X)± SD	Standard deviasi (SD)p-value
1	S-ECC	8	6.2525 ± 0.64482	p= 0.00000.64482
2	Free Caries	8	8.4138 ±1.10397	1.10397

In Ttable 1 shows that the mean expression of T lymphocytes (CD4 +) in S-ECC higher than caries free children.

Table 2. Test for normality of T lymphocyte (CD4 +) cell expression after incubation 24 hours analyzed by Flow Cytometry

variabel	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CD4+	.122	16	0.200	0.940	16	.345

Data normality test using shapiro Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t test

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Table 3. Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC group and free caries using the independent t test

No	Group			
	S-ECC		Free Caries	
	Variabel	Mean Difference	Std. Error Difference	Sig. (2-tailed)
1	CD4	-2.16125	.45201	.000

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Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC and free caries groups showed a p-value of 0,000, which is smaller than 0.05 (p <0.05), which means that there are significant differences between the S-ECC and free caries groups

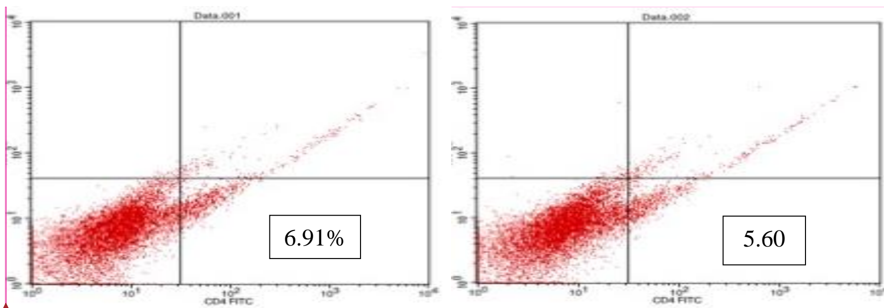

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva ofthe Free Caries Free

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of Free Caries Free

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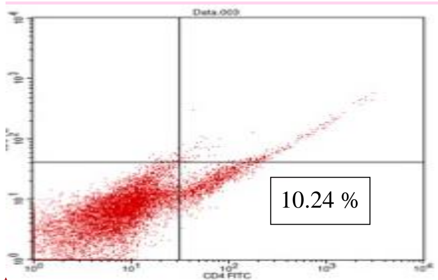

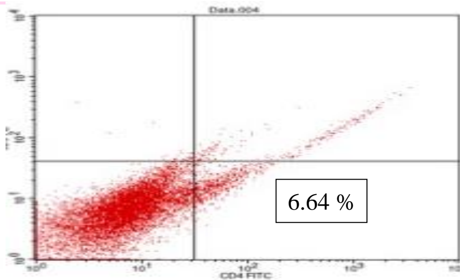

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

DISCUSSION

Steptococcus mutans (*S. mutans*) is the main bacterium that has a strong relationship with ECC while other oral bacteria in dental biofilms can be involved in the initiation and development of caries.[16] ~~(Hajishengallis et. Al., 2017).~~ Other bacteria associated with ECC are the Lactobacillus species which play an important role in the development of lesions.[17] ~~(Li and Tanner, 2015).~~ Actinomyces species, especially *Actinomyces gerencseriae*, are also associated with caries initiation. in addition, some non-mutans streptococci that have acidogenic and aciduric~~akidurik~~ properties are also associated with dental caries. Epidemiological data indicate that in the pathogenesis of dental caries, *Candida albicans* also plays an active role.[18] ~~Sukuraman and Pradeep, 2014).~~

T lymphocyte cells (CD4 +), known as helper T cells, are effector cells for cell-mediated immunity. T lymphocytes (CD4 +) are naive and must be activated to start effector functions, this activation occurs through interactions with professional "antigen-presenting cells (pro-APC) especially dendritic cells that lead to intracellular pathways that regulate T cell receptors (TCR) more specifically against antigens in T cells.

TCR and its co-receptors, such as CD4, form complexes with class 2 MHC receptors and antigens. CD4 + lymphocyte cells are then activated and produce cytokines to start the

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immune response of leukocyte cells or other immune cells of cell-mediated immunity and activate humoral immunity branches that depend on T cells, then CD4 + T cells recognize protein antigens and activate B cells to produce immunoglobulins in response to antigens. [~~(~~19,20~~4,15)~~]. ~~(Shen et. al., 2019, Bourne et. al., 2019).~~

The results of the study as shown in Table 1 show that the expression of T lymphocytes (CD4 +) cells in S-ECC is significantly lower than in free caries, this may cause the high *S. mutans* bacteria found in S-ECC saliva cannot be in

~~acquisition~~ a~~A~~cquisition by adaptive immunity because TCR and its co-receptors, such as CD4 which ~~have the ability to~~can form complexes with class 2 major receptor histocompatibility complex (MHC) receptors and antigens, cannot function optimally so that quantitatively the number of *S. mutans* which are bacteria that causes caries is higher ~~,~~ compared to caries-free children. [~~(~~21~~16)~~]. ~~(Lutfi et al., 2015).~~ Expression of T lymphocytes (CD4 +) in S-ECC causes the release of pro-inflammatory cytokines that function as ~~chemoatractants~~chemoattractant of neutrophil cells, because the movement of neutrophils toward the infection area is less than optimal, the movement of macrophages is also less than optimal towards the area of infection, giving *S. mutans* the opportunity to develop and do damage to the teeth

In addition to the above, the low expression of CD4 + T lymphocyte cells in S-ECC ~~causes~~ results in slow B cells ~~to~~ forming antibodies ~~to slow~~. This happens because CD4 + T cells recognize antigens well and can activate B cells to produce antibodies in the form of immunoglobulins in response to *S. mutans* antigens.

CONCLUSION

Low T lymphocyte (CD4$^+$) expression in S-ECC may be one of the causes of S-ECC

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~~Acknowledgment~~

~~The authors would like to thank Prof. Muhaimin Rifa'i, PhD.Med.Sc for the help in conducting this research.~~

~~Financial support and sponsorship~~

~~The authors would like to thank Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the grant funding provided for this research.~~

~~Conflicts of interest~~

~~The authors of this manuscript declare that they have no coflicts of interest, real or perceived, nancial or non- nancial in this article~~

REFERENCES

1. Naidu R, Nunn J, Donnelly-Swift E. Oral health-related quality of life and early childhood caries among preschool children in Trinidad. BMC Oral Health ~~[Internet].~~ 2016;16(1):1–9. ~~Available from: http://dx.doi.org/10.1186/s12903-016-0324-7~~
2. Abanto J, Carvalho TS, Mendes FM, ~~Wanderley MT, Bonecker M, Raggio DP~~et al. Impact of oral diseases and disorders on oral health-related quality of life of preschool children. Community Dent Oral Epidemiol ~~.~~ 2011 ~~Apr~~;39(2):105–14.
3. Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E. Beyond the dmft: the

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human and economic cost of early childhood caries. J Am Dent Assoc~~.~~ 2009 Jun;140(6):650–7.

4. Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected healthy people 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief~~.~~ 2012;(104):1–8.

5. Dye BA, Hsu K-LC, Afful J. Prevalence and Measurement of Dental Caries in Young Children. Pediatr Dent~~.~~ 2015;37(3):200–16.

6~~5b~~. Amalia R. Indonesia. In: Folayan MO (ed). A Compendium on Oral Health of Children Around the World: Early Childhood Caries. New York, NY: Nova Biomedical; ~~(~~2018~~)~~. ~~p. 155–65.~~

7~~5c~~. ~~Rahel Wahjuni~~ Sutjipto RW, ~~Herawati~~ Herawati H, ~~Satiti~~ Kuntari S~~, 2014.~~. The prevalences of early childhood caries and severe early childhood caries in preschool children at Gunung Anyar Surabaya~~)~~. Dent J 2014;47 ??? ~~mkg.v47.i4.p186~~ 186-189

8~~6~~. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med ~~[Internet].~~ 2013 ~~Jan~~;4(1):29–38. ~~Available from: https://www.ncbi.nlm.nih.gov/pubmed/23633832~~

9~~7~~. Liang Y, Chen X, Chen J, Liu F. Differentiation and function of t cell subsets in infectious diseases. J Immunol Res~~.~~ 2018;2018:2–4.

10~~8~~. Zhen A, Krutzik SR, Levin BR, ~~Kasparian S, Zack JA, Kitchen SG~~et al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. J Virol~~.~~ 2014~~; Sep;~~88(17):9934–46.

11~~9~~. Artyomov MN, Lis M, Devadas S, ~~Davis MM, Chakraborty AK~~et al. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. Proc Natl Acad Sci

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USA~~-S-A.~~ 2010;107(39):16916–21.

120. Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature ~~[Internet].~~ 1987;330(6145):256–9. ~~Available from: https://doi.org/10.1038/330256a0~~

13. Jonsson P, Jennifer H, Southcombe, ~~Santos AM, Huo J~~ et al.~~2016.~~ Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USAA~~A~~ 2016;113(20): 5682–5687.

14. Luthfi M, Setijanto D, Rahardjo MB, ~~Indrawati R, Rachmadi P, Ruth MS~~et al.~~(2019)~~. Correlation between human neutrophil peptide 1–3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J 2019;16:81-6

15. Luthfi M, Oki AS, Indrawati R, -Latuamury NS~~ (2019)~~. Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries-Free in Surabaya. Malaysian Journal of Medicine and Health Sciences 2019;~~Vol.~~15~~ Supp~~(-3):8-10.

161. Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Mol Oral Microbiol ~~[Internet].~~ 2017~~ Feb 1~~;32(1):24–34. ~~Available from: https://doi.org/10.1111/omi.12152~~

172. Li Y, Tanner A. Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries. Pediatr Dent~~.~~ 2015;37(3):226–44.

183. Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. Front Pediatr~~.~~ 2017;5~~(July)~~:1–7.

194. Shen H, Wu N, Nanayakkara G, ~~Fu H, Yang Q, Yang WY,~~ et al. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci ~~(~~Landmark Ed. 2019~~ Jan~~;24:96–132.

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20~~15~~. Bourne N, Perry CL, Banasik BN, ~~Miller AL, White M, Pyles RB,~~ et al. ~~crossm~~ Increased Frequency of Virus Shedding by Herpes Simplex Virus 2-Infected Guinea Pigs in the Absence of CD4^{+} T Lymphocytes. J Virol ~~.~~2019;93(4):1–11.

21~~16~~. Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah Streptococcus mutans (S. mutans) dan Level Ekspresi Interlukin 8 (IL-8) pada Severe Early Childhood Caries. Maj Kedokt Gigi Indones~~.~~ 2015;20(2):142.

~~Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proc Natl Acad Sci U S A.* (2010) 107:16916–21. doi: 10.1073/pnas.1010568107~~

~~Bourne N, Perry CL, Banasik BN, Miller AL, White M, Pyles RB, Schäfer H, Milligan GN. Increased Frequency of Virus Shedding by Herpes Simplex Virus 2-Infected Guinea Pigs in the Absence of CD4^{+} T Lymphocytes. J. Virol. 2019 Feb 15;93(4) [PMC free article] [PubMed]~~

~~Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013 Jan;4(1):29–38.~~

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Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected Healthy People 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief. 2012 Aug;104:1–8.

Dye BA, Hsu KL, Afful J. Prevalence and measurement of dental caries in young children. Pediatr Dent. 2015 May-Jun;37(3):200–16.

R.Naidu,J.Nunn,andE.Donnelly-Swift,"Oralhealth-related quality of life and early childhood caries among preschool children in Trinidad," *BMC Oral Health*, vol. 16, no. 1, p. 128, 201

Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature*. (1987) 330:256–9. doi: 10.1038/330256a0

Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Molecular Oral Microbiology. 2017;**32**(1):24-34

J. Abanto, T. S. Carvalho, F. M. Mendes, M. T. Wanderley, M. Bonecker, and D. P. Raggio, "Impact of oral diseases and disorders on oral health-related quality of life of preschool children," *Community Dentistry and Oral Epidemiology*, vol. 39, no. 2, pp. 105–114, 2011.

Jonsson P, Southcombe JH, Santos AM, Huo J, Fernandes RA, McColl J, et al. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein

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interactions. *Proc Natl Acad Sci U S A.* (2016) 113:5682–7. doi: 10.1073/pnas.1513918113 10.10841513918113 10.1084/jem.20122528

Li Y, Tanner A. E ect of antimicrobial intervention on oral microbiota associated with early childhood caries. Pediatric Dentistry. 2015;37(3):226-244

Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah *Streptococcus mutans (S. mutans)* dan level ekspresi interlukin 8 (IL-8) pada severe early childhood caries. Maj Ked Gi Ind 2015;1:1422015; 1:142–8.

P. S. Casamassimo, S. ikkurissy, B. L. Edelstein, and E. Maiorini, “Beyond the dmft: the human and economic cost of early childhood caries,” *Journal of the American Dental Association*, vol. 140, no. 6, pp. 650–657, 2009.

Sukuraman A, Pradeep SA. Early childhood caries: Prevalence, risk factors, and preven- tion. Frontiers in Pediatrics. 2017;5:1572017; 5:157

Shen H, Wu N, Nanayakkara G, Fu H, Yang Q, Yang WY, Li A, Sun Y, Drummer Iv C, Johnson C, Shao Y, Wang L, Xu K, Hu W, Chan M, Tam V, Choi ET, Wang H, Yang X. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci (Landmark Ed). 2019 Jan 01;24:96-132.

Yan Li, Jibieke Wulaerhan, Yuan Liu, Ayinuer Abudureyimuand Jin Zhao. 2017. Prevalence of severe early childhood caries and associated socioeconomic and behavioral factors in Xinjiang, China: a cross-sectional study. BMC Oral Health 17:144 .1-10

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Yuejin Liang, XiaojunLiang, Xiaojun Chen, Jinling Chen, Fengliang Liu, 2018. Differentiation and Function of T Cell Subsets in Infectious Diseases. Journal of Immunology Research Volume 2018. p1-2

Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen S, et al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. *J Virol.* (2014) 88:9934–46.

10b. Jonsson P, Jennifer H, Southcombe, Santos AM, Huo J et al.2016. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USA 113(20): 5682–5687

10c. Luthfi M, Setijanto D, Rahardjo MB, Indrawati R, Rachmadi P, Ruth MS.(2019). Correlation between human neutrophil peptide 1–3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J;16:81–6

10d. Luthfi M, Oki AS, Indrawati R Latuamury NS (2019). Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries Free in Surabaya. Malaysian Journal of Medicine and Health Sciences Vol.15 Supp 3:8-10

Caption figure :

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva of Caries Free

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of Caries Free

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC

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Analysis of lymphocyte t (cd4 +) cell expression on severe early childhood caries and free caries

Muhammad Luthfi[1], Priyawan Rachmadi[2], Aqsa Sjuhada Oki[3], Retno Indrawati[4], Agung Sosiawan[5]

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Acknowledgements :

The authors would like to thank Prof. Muhaimin Rifa'i, PhD.Med.Sc for the help in conducting this research.

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~~**Title**~~

Key words: Severe early childhood caries, adaptive immunity, lymphocyte T (CD4+) cells expression

Acknowledgements:

Correspondence:

Contributions:

Conflicts of interest

The authors of this manuscript declare that they have no coflicts of interest, real or perceived, nancial or non- nancial in this article

Conflict of Interests:

Funding:

The authors would like to thank Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research,

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ANALYSIS OF LYMPHOCYTE T (CD4 +) CELL EXPRESSION ON SEVERE EARLY CHILDHOOD CARIES AND FREE CARIES FREE

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ABSTRACT

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Background: Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. Cariogenic bacteria are a significant risk factor for ECC

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associated with early colonization and high levels of cariogenic microbes (Streptococcus mutans (*S. mutans*). ~~lymphocyte~~ Lymphocyte T ($CD4^+$) cells known as helper T cells, are effector cells for mediated host immunity. ~~N~~Naive T cells ($CD4^+$) must be activated to initiate effector function.~~,~~ T~~t~~his activation occurs through interaction with professional antigen-presenting cells (pro-APC), especially dendritic cells that lead to intracellular pathways that regulate T cell receptor (TCR) more specifically against antigen in T cells.

~~**Material and method:**~~ Lymphocyte cells from samples were collected from severe early childhood caries (S-ECC) and Free caries aged 5 to 6 years. The subjects were instructed to gargle 10 ml of sterile NaCl 1.5% solution for 30 seconds, and expectorate it into a sterile glass then analyzing T lymphocyte cell (CD4 +) expression using flow cytometry.

~~**Results:**~~ L~~l~~ymphocyte T ($CD4^+$) cell expression at S-ECC (6.2525 ±, 64482) while in free caries (8.4138 ± 1.10397) with p-value (p = 0. 000). Conclusion

~~**Conclusion:**~~ of lymphocyte T ($CD4^+$) cells e~~E~~xpression at S-ECC is lower than that occurring in free caries.~~s~~

~~**Key words**: *Severe Early Childhood Caries, adaptive immunity, lymphocyte T ($CD4^+$) cells Expression*~~

~~PENDAHULUA~~INTRODUCTION~~N~~

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Early childhood caries (ECC) is still one of the many diseases found in children throughout the world. ECC does not only affect the oral health of children, but also general body health.[(1)] ECC not only involves pain in the oral cavity, orthodontic problems, and damage to the enamel, but can also cause problems with food intake, speech and increased risk for caries development in permanent teeth.[(2)]. (Abanto et al., 2016). Early loss of primary teeth often leads to orthodontic problems in adult life .[(3)].(Casamassimo et. al., 2009). Early childhood caries (ECC) is the most common childhood chronic disease, with almost 1.8 billion new cases per year globally[(4)] [Dye et al., 2012) which occurs in about 37% of children aged 2-5 years in America States (Dye et al., 2012) and up to 73% of preschoolers who are socially economically disadvantaged in developing and industrialized countries.[(5)]. ECC is also highly prevalence in preschool children living in developing countries like Indonesia [(6 5b6)] the prevalence of ECC in group of children aged 6 months - 3 years at Gunung Anyar Surabaya-Indonesaia was 30.8 % , while the prevalence was 29.2 % SECC. [(7 5c)].

……

[Dye et al., 2015]. ECC was defined as the presence of ≥1 decay, loss (due to caries), or full tooth surface in primary teeth in children 71 months of age or younger. S-ECC occurs in children <3 years with ≥1 rot, missing (due to caries), or full tooth surface and in children aged 4-6 years with high caries score.[(86)] (Colak et al., 2013). ECC and S-ECC remain serious problems that occur in school children in Xinjiang. Lower sociodemographic status (disadvantaged areas, low-educated mothers, low-income families, caregivers with cavities), risky dietary behavior (consumption of high frequency sweets, frequent meals before going to bed), oral hygiene behaviors that are at risk of ECC such as at what age start to brush teeth risky oral hygiene behaviors (starting to brush teeth) at an age of age older), aand use of

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dental services (past dental visits, parents who have received oral health care instructions) are associated with an increased risk of ECC and S-ECC.

Severe early childhood caries (S-ECC) is an infectious disease that is a public health problem in the world, in spite of ongoing control efforts. The purpose of the host immune response during infection is to clear pathogens that attack with limited tissue damage. Both innate cells and adaptive T cells play a key role in clearing pathogens directly through the release of proinflammatory cytokines and the activity of cytotoxic T lymphocytes (CTL). In addition, helper (Th) T cells and regulatory Treg cells are required for antibodies secreted by plasma cells and immunomodulatory cytokines (eg, IL-10), respectively. In recent years, the role of the new set of Th cells, including follicular T cells namely Th17, Th22, in regulating anti-infective immunity, has become very important, because they play an important role in the development and outcome of disease.[97]

Cluster of differentiation 4 (CD4) coreceptor expressed in a subset of T cells, plays a role in differentiation, migration and cytokine expression.[108] T cells involved in antigen recognition, CD4 stabilizes the ternary complex pMHC-TCR and CD4 recruits Lck kinase to phosphorylate ITAM and initiate intracellular signaling during activation of T cells induced by antigens.[119] CD4 was originally described as an adhesion molecule that enhances contact between T cells and precenting cell antigens. In their pillar work, Doyle and Strominger found direct correlations of other specific T cells involved in interactions.[120] CD4 binds MHCII molecules with very low 3D affinity.[13] Based on the above background, the researchers wanted to analyze how the expression of T lymphocytes (CD4+) cells in S-ECC and caries-free.

MATERIAL AND METHODS

This study was an analytic observational study, with cross-sectional analysis on two groups of sample; children with S-ECC and free caries children. All the procedures in this study had been reviewed and approved by the Health Research Ethical Clearance Commission of Universitas Airlangga, Faculty of Dental Medicine, with certificate no 209/HRECC. FODM/IX/2017.

Lymphocyte Isolation

Lymphocyte cells from saliva obtained by instructing the subject to rinse with 10 ml of 1.5% sterile NaCl solution while rinsing, but not swallowed for 30 seconds, then expectorated in sterile glass. This procedure was repeated 4 times. The sample was then centrifuged at 450g for 15 minutes, at 40C. The centrifugation pellets were then mixed with 2 ml of RPMI medium, then the samples were vortexed. ~~(Gasparoto et al., 2011).14~~ The results of the filter in the form of cell suspension are then calculated using a hemocytometer.

The same volume of cell suspension and 0.2% dye of trypan blue were mixed in the eppendorf tube and in doing vortex ~~divortex.~~ The same suspension aliquots (20 µl) were added to both chamber haemocytometers and observed under a microscope (10X objective). The mixture is withdrawn with capillary action. The cells are counted in an area of 16 squares which is equivalent to the number of cells x104 / ml. Only translucent cells are counted in the box. The number of cells per ml is calculated using the following formula:

Cell / ml = average number of cells per primary square x 10^4 x dilution factor

Lymphocyte Culture and Cultivation

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Lymphocyte cells ($3x10^5$cells/ml) were cultured in the tissue culture flask (Greiner) 75cm^2 with complete culture medium (RPMI-1640, 10% fetal calf serum (FCS), and 1% penicillin/streptomycin) in 5% CO2 and atmosphere humidity 95% at 37°C for 24 hours. The cultures were checked daily to observe the changes in color, turbidity, density, and growth pattern using inverted light microscope (Nikon

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CD4⁺ Expression Analysis

The expression of $CD4^+$ were observed by means of flow cytometry method adapted from. ~~(~~(15 ~~Cherng et al (2008)~~). Fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Peridinin chlorophyll protein (PerCP), PerCP-Cy5.5-conjugated monoclonal antibodies (mAbs) from Becton Dickinson (San Jose, CA, USA). The optimum concentration of mAbs were determined for each mAb by means of titration. Flow cytometry can both measure and analyze the physical characteristics of a particle such as cell since it can flow into the fluid stream through the light. The light scattered by the cell can be used to analyze changes in size, granularity, internal complexity, and relative fluorescence intensity ~~(Zgonc dan Gruber 1998)~~. Flow cytometry analysis is conducted to discover the immunomodulatory pattern of lymphocyte using conjugated monoclonal antibody.

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Salivary lymphocytes were moved into FACS tube and washed with 4ml Dulbecco Phosphate Buffer Saline (DPBS~~), and~~) and centrifuged for 5 minutes at 2000rpm; the supernatant was subsequently removed. The pellet in DPBS were once again washed and centrifuged at 1800 rpm for 8 minutes. The cells were stained using yellow viability dye (1ml stain/1000µl DPBS) then vortexed and incubated at 4°C for 15 minutes. The cells were subsequently washed with 4ml DPBS and 1% FCS, centrifuged at 1800 rpm for 8 minutes and the supernatant was removed. The cells were stained with the exact required volume of mAbs, followed by vortexed and incubated in refrigerator for 20 minutes. After washed in cold

DPBS and 1% FCS, cells were centrifuged for 8 minutes at 1800 rpm and the supernatant were removed. The cells were once again vortexed and 100µl of reagent A was added into the sample and cooled for 10 minutes. 50µl of mixture that had been fixated in reagent A was added into each ~~samples, and~~samples and covered with aluminum foil and stored in refrigerator until acquisition at LSR2 flow cytometry.

The stained lymphocytes were analyzed using flow cytometer (LSR 11 Sorvall RT7 Plus, Becton Dickinson, USA) with cell quest software (Becton Dickinson, USA). The results were analyzed using flow Jo 7.0 (USA) software. The expression of $CD8^+$ were analyzed using standard FACScan procedure with mAbs according to the producer protocol. The results are calculated and presented in mean.

Statistical analysis

The acquired data was analyzed the normality and homogeny, then followed by T-test to find the difference between two groups, with the level of significance at 0.05.

RESULT

Data normality test using shapiro-Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t tes.

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Table 1. Mean and standard deviation of the expression of T lymphocytes (CD4 +) after 24-hour incubation were analyzed by flow cytometry test and statistical test t

No	Group	N	CD4^{+} eExpression	
			Mean (X)± SD	Standard deviasi (SD)p-value
1	S-ECC	8	6.2525 ± 0.64482	p= 0.00000.64482
2	Free Caries	8	8.4138 ±1.10397	1.10397

In Ttable 1 shows that the mean expression of T lymphocytes (CD4 +) in S-ECC higher than caries free children.

Table 2. Test for normality of T lymphocyte (CD4 +) cell expression after incubation 24 hours analyzed by Flow Cytometry

variabel	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CD4^{+}	.122	16	0.200	0.940	16	.345

Data normality test using shapiro Wilk obtained p value of expression of T lymphocytes (CD4 +) of 0.200 while the value of p value of CD4 of 0.345 shows that both p-values > 0.05 which means the data are normally distributed, because the data are normally distributed then a comparative test is then performed a comparative test between groups using the independent t test

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Table 3. Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC group and free caries using the independent t test

No	Group			
	S-ECC		Free Caries	
	Variabel	Mean Difference	Std. Error Difference	Sig. (2-tailed)
1	CD4	-2.16125	.45201	.000

Comparative test results of T lymphocyte (CD4 +) cell expression between the S-ECC and free caries groups showed a p-value of 0,000, which is smaller than 0.05 (p <0.05), which means that there are significant differences between the S-ECC and free caries groups

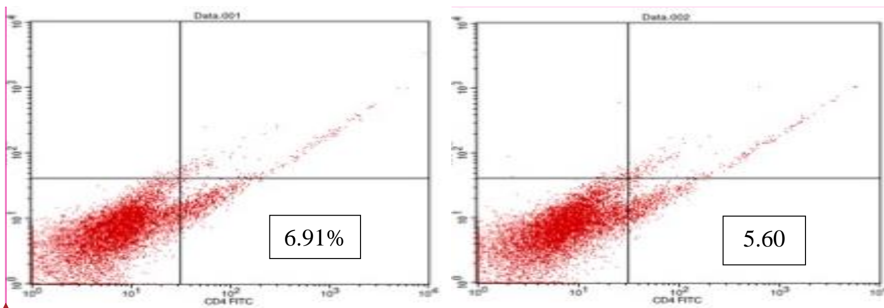

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva ofthe Free Caries Free

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of Free Caries Free

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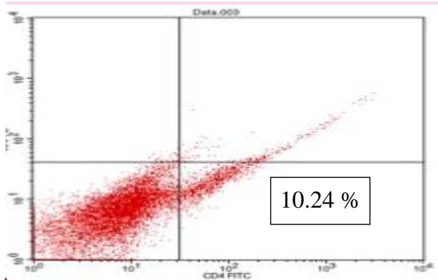

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

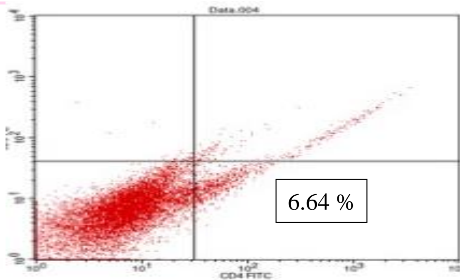

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

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DISCUSSION

Steptococcus mutans (*S. mutans*) is the main bacterium that has a strong relationship with ECC while other oral bacteria in dental biofilms can be involved in the initiation and development of caries.[16] ~~(Hajishengallis et. Al., 2017).~~ Other bacteria associated with ECC are the Lactobacillus species which play an important role in the development of lesions.[17] ~~(Li and Tanner, 2015).~~ Actinomyces species, especially *Actinomyces gere~~n~~cseriae*, are also associated with caries initiation. in addition, some non-mutans streptococci that have acidogenic and aciduric~~akidurik~~ properties are also associated with dental caries. Epidemiological data indicate that in the pathogenesis of dental caries, *Candida albicans* also plays an active role.[18] ~~Sukuraman and Pradeep, 2014).~~

T lymphocyte cells (CD4 +), known as helper T cells, are effector cells for cell-mediated immunity. T lymphocytes (CD4 +) are naive and must be activated to start effector functions, this activation occurs through interactions with professional "antigen-presenting cells (pro-APC) especially dendritic cells that lead to intracellular pathways that regulate T cell receptors (TCR) more specifically against antigens in T cells.

TCR and its co-receptors, such as CD4, form complexes with class 2 MHC receptors and antigens. CD4 + lymphocyte cells are then activated and produce cytokines to start the

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immune response of leukocyte cells or other immune cells of cell-mediated immunity and activate humoral immunity branches that depend on T cells, then CD4 + T cells recognize protein antigens and activate B cells to produce immunoglobulins in response to antigens. [(19,204,15)]. ~~(Shen et. al., 2019, Bourne et. al., 2019).~~

The results of the study as shown in Table 1 show that the expression of T lymphocytes (CD4 +) cells in S-ECC is significantly lower than in free caries, this may cause the high *S. mutans* bacteria found in S-ECC saliva cannot be in

~~acquisition~~ aAcquisition by adaptive immunity because TCR and its co-receptors, such as CD4 which ~~have the ability to~~can form complexes with class 2 major receptor histocompatibility complex (MHC) receptors and antigens, cannot function optimally so that quantitatively the number of *S. mutans* which are bacteria that causes caries is higher compared to caries-free children. [(2116)]. ~~(Lutfi et al., 2015).~~ Expression of T lymphocytes (CD4 +) in S-ECC causes the release of pro-inflammatory cytokines that function as ~~chemoatractants~~chemoattractant of neutrophil cells, because the movement of neutrophils toward the infection area is less than optimal, the movement of macrophages is also less than optimal towards the area of infection, giving *S. mutans* the opportunity to develop and do damage to the teeth

In addition to the above, the low expression of CD4 + T lymphocyte cells in S-ECC ~~causes~~ results in slow B cells ~~to~~ forming antibodies ~~to slow~~. This happens because CD4 + T cells recognize antigens well and can activate B cells to produce antibodies in the form of immunoglobulins in response to *S. mutans* antigens.

CONCLUSION

Low T lymphocyte ($CD4^+$) expression in S-ECC may be one of the causes of S-ECC

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Acknowledgment

The authors would like to thank Prof. Muhaimin Rifa'i, PhD.Med.Sc for the help in conducting this research.

Financial support and sponsorship

The authors would like to thank Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the grant funding provided for this research.

Conflicts of interest

The authors of this manuscript declare that they have no coflicts of interest, real or perceived, nancial or non- nancial in this article

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REFERENCES

1. Naidu R, Nunn J, Donnelly-Swift E. Oral health-related quality of life and early childhood caries among preschool children in Trinidad. BMC Oral Health [Internet]. 2016;16(1):1–9. Available from: http://dx.doi.org/10.1186/s12903-016-0324-7

2. Abanto J, Carvalho TS, Mendes FM, Wanderley MT, Bonecker M, Raggio DPet al. Impact of oral diseases and disorders on oral health-related quality of life of preschool children. Community Dent Oral Epidemiol . 2011 Apr;39(2):105–14.

3. Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E. Beyond the dmft: the

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human and economic cost of early childhood caries. J Am Dent Assoc~~.~~ 2009 Jun;140(6):650–7.

4. Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected healthy people 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief~~.~~ 2012;(104):1–8.

5. Dye BA, Hsu K-LC, Afful J. Prevalence and Measurement of Dental Caries in Young Children. Pediatr Dent~~.~~ 2015;37(3):200–16.

6~~5b~~. Amalia R. Indonesia. In: Folayan MO (ed). A Compendium on Oral Health of Children Around the World: Early Childhood Caries. New York, NY: Nova Biomedical; ~~(~~2018~~)~~. ~~p. 155–65.~~

7~~5c~~. ~~Rahel Wahjuni~~ Sutjipto RW, ~~Herawati~~ Herawati H, ~~Satiti~~ Kuntari S~~, 2014.~~. The prevalences of early childhood caries and severe early childhood caries in preschool children at Gunung Anyar Surabaya~~)~~. Dent J 2014;47 ??? ~~mkg.v47.i4.p186~~ 186-189

8~~6~~. Colak ~~-~~H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med ~~[Internet].~~ 2013 ~~Jan~~;4(1):29–38. ~~Available from: https://www.ncbi.nlm.nih.gov/pubmed/23633832~~

9~~7~~. Liang Y, Chen X, Chen J, Liu F. Differentiation and function of t cell subsets in infectious diseases. J Immunol Res~~.~~ 2018;2018:2–4.

10~~8~~. Zhen A, Krutzik SR, Levin BR, ~~Kasparian S, Zack JA, Kitchen SG~~et al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. J Virol~~.~~ 2014~~; Sep;~~88(17):9934–46.

11~~9~~. Artyomov MN, Lis M, Devadas S, ~~Davis MM, Chakraborty AK~~et al. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. Proc Natl Acad Sci

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USA~~ S A. ~~2010;107(39):16916–21.

12~~0~~. Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature ~~[Internet]. ~~1987;330(6145):256–9. ~~Available from: https://doi.org/10.1038/330256a0~~

13. Jonsson P, Jennifer H, Southcombe, ~~Santos AM, Huo J ~~et al.~~2016.~~ Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USA~~A~~ 2016;113(20): 5682–5687.

14. Luthfi M, Setijanto D, Rahardjo MB, ~~Indrawati R, Rachmadi P, Ruth MS~~et al.~~(2019)~~. Correlation between human neutrophil peptide 1–3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J 2019;16:81-6

15. Luthfi M, Oki AS, Indrawati R, ~~-~~Latuamury NS~~ (2019)~~. Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries-Free in Surabaya. Malaysian Journal of Medicine and Health Sciences 2019;~~Vol.~~15~~ Supp~~(-3):8-10.

16~~1~~. Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Mol Oral Microbiol ~~[Internet].~~ 2017~~ Feb 1~~;32(1):24–34. ~~Available from: https://doi.org/10.1111/omi.12152~~

17~~2~~. Li Y, Tanner A. Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries. Pediatr Dent~~.~~ 2015;37(3):226–44.

18~~3~~. Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. Front Pediatr~~.~~ 2017;5~~(July)~~:1–7.

19~~4~~. Shen H, Wu N, Nanayakkara G, ~~Fu H, Yang Q, Yang WY, ~~et al. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci ~~(~~Landmark Ed. 2019~~ Jan~~;24:96–132.

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2015. Bourne N, Perry CL, Banasik BN, Miller AL, White M, Pyles RB, et al. crossm Increased Frequency of Virus Shedding by Herpes Simplex Virus 2-Infected Guinea Pigs in the Absence of CD4+ T Lymphocytes. J Virol .2019;93(4):1–11.

2116. Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah Streptococcus mutans (S. mutans) dan Level Ekspresi Interlukin 8 (IL-8) pada Severe Early Childhood Caries. Maj Kedokt Gigi Indones. 2015;20(2):142.

Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proc Natl Acad Sci U S A.* (2010) 107:16916–21. doi: 10.1073/pnas.1010568107

Bourne N, Perry CL, Banasik BN, Miller AL, White M, Pyles RB, Schäfer H, Milligan GN. Increased Frequency of Virus Shedding by Herpes Simplex Virus 2-Infected Guinea Pigs in the Absence of CD4+ T Lymphocytes. J. Virol. 2019 Feb 15;93(4) [PMC free article] [PubMed]

Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013 Jan;4(1):29–38.

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Dye BA, Li X, Thorton-Evans G. Oral health disparities as determined by selected Healthy People 2020 oral health objectives for the United States, 2009-2010. NCHS Data Brief. 2012 Aug;104:1–8.

Dye BA, Hsu KL, Afful J. Prevalence and measurement of dental caries in young children. Pediatr Dent. 2015 May-Jun;37(3):200–16.

R.Naidu,J.Nunn,andE.Donnelly-Swift,"Oralhealth-related quality of life and early childhood caries among preschool children in Trinidad," *BMC Oral Health*, vol. 16, no. 1, p. 128, 201

Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature.* (1987) 330:256–9. doi: 10.1038/330256a0

Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Molecular Oral Microbiology. 2017;**32**(1):24-34

J. Abanto, T. S. Carvalho, F. M. Mendes, M. T. Wanderley, M. Bonecker, and D. P. Raggio, "Impact of oral diseases and disorders on oral health-related quality of life of preschool children," *Community Dentistry and Oral Epidemiology*, vol. 39, no. 2, pp. 105–114, 2011.

Jonsson P, Southcombe JH, Santos AM, Huo J, Fernandes RA, McColl J, et al. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein

interactions. *Proc Natl Acad Sci U S A.* (2016) 113:5682–7. doi: 10.1073/pnas.1513918113 10.10841513918113 10.1084/jem.20122528

Li Y, Tanner A. E ect of antimicrobial intervention on oral microbiota associated with early childhood caries. Pediatric Dentistry. 2015;37(3):226-244

Luthfi M, Indrawati R, Arundina I, Dachlan YP. Korelasi Jumlah *Streptococcus mutans (S. mutans)* dan level ekspresi interlukin 8 (IL-8) pada severe early childhood caries. Maj Ked Gi Ind 2015;1:1422015; 1:142–8.

P. S. Casamassimo, S. ikkurissy, B. L. Edelstein, and E. Maiorini, "Beyond the dmft: the human and economic cost of early childhood caries," *Journal of the American Dental Association*, vol. 140, no. 6, pp. 650–657, 2009.

Sukuraman A, Pradeep SA. Early childhood caries: Prevalence, risk factors, and preven- tion. Frontiers in Pediatrics. 2017;5:1572017; 5:157

Shen H, Wu N, Nanayakkara G, Fu H, Yang Q, Yang WY, Li A, Sun Y, Drummer Iv C, Johnson C, Shao Y, Wang L, Xu K, Hu W, Chan M, Tam V, Choi ET, Wang H, Yang X. Co-signaling receptors regulate T-cell plasticity and immune tolerance. Front Biosci (Landmark Ed). 2019 Jan 01;24:96-132.

Yan Li, Jibieke Wulaerhan, Yuan Liu, Ayinuer Abudureyimuand Jin Zhao. 2017. Prevalence of severe early childhood caries and associated socioeconomic and behavioral factors in Xinjiang, China: a cross-sectional study. BMC Oral Health 17:144 .1-10

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Yuejin Liang, XiaojunLiang, Xiaojun Chen, Jinling Chen, Fengliang Liu, 2018. Differentiation and Function of T Cell Subsets in Infectious Diseases. Journal of Immunology Research Volume 2018. p1-2

Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen S, et al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. *J Virol.* (2014) 88:9934–46.

10b. Jonsson P, Jennifer H, Southcombe, Santos AM, Huo J et al.2016. Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. Proc Nati Acad Sci USA 113(20): 5682–5687

10c. Luthfi M, Setijanto D, Rahardjo MB, Indrawati R, Rachmadi P, Ruth MS.(2019). Correlation between human neutrophil peptide 1-3 secretion and azurophilic granule (CD63) expression in early childhood caries. Dent Res J;16:81-6

10d. Luthfi M, Oki AS, Indrawati R Latuamury NS (2019). Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries Free in Surabaya. Malaysian Journal of Medicine and Health Sciences Vol.15 Supp 3:8-10

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Caption figure :

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva of Caries Free

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of Caries Free

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC

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