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 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 (~~65b6~~) 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. (~~75c~~).

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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

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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.

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., 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.

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

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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

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

$CD4^+$ 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

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= 0.00000.64482 1.10397
2	Free Caries	8	8.4138 ±1.10397	

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

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
variabel	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-

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~~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~~

~~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

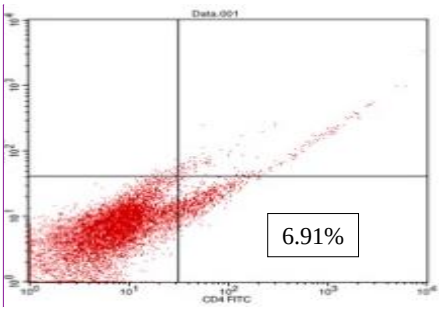

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva of~~the~~ ~~Free~~ Caries Free

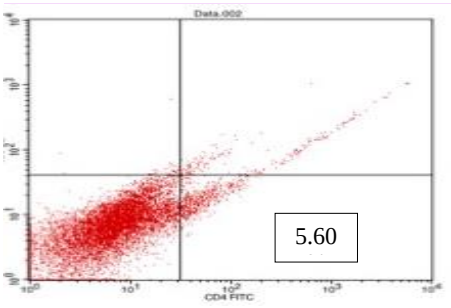

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the saliva of ~~Free~~ Caries Free

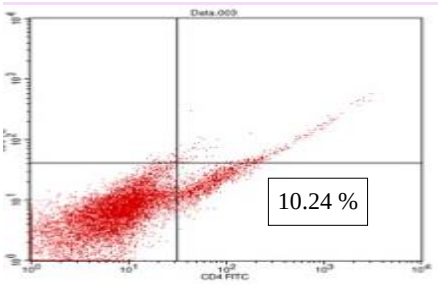

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

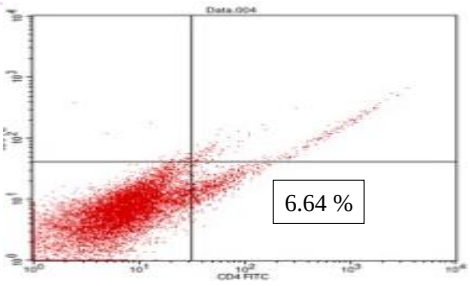

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

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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~~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~~akiduric~~ 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

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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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. 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.

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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.
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, 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*
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. 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. 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.
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 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

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

19. 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.

20. 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.

21. 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

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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INSBIOMM_Abstract Revised

INSBIOMM Conference <insbiomm@gmail.com> 29 Juli 2019 09.06
Kepada: m.luthfi@fkg.unair.ac.id

Dear Author

Please revised the abstract "Difference of Expression Of Limfosit T (Cd4 +) Cells In Severe Early Childhood Caries And Free Caries" and "EFFECT OF ADMINISTERING OKRA FRUIT (Abelmoschus esculentus) EXTRACT IN ACCELERATING WOUND HEALING THROUGH INCREASING FIBROBLAST CELL EXPRESSION".

The revised itself is due: 2019-08-05

Thank you for considering this request

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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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~~**Title**~~

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

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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

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

Conference presentation:

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

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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).

.....

[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. [8] (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

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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., 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.

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% 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

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.

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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

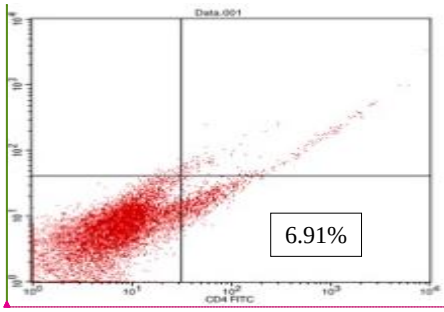

Figure 1. T lymphocyte (CD4 +) cells expression (6.91%) in the saliva of~~the~~ ~~Free~~ Caries Free

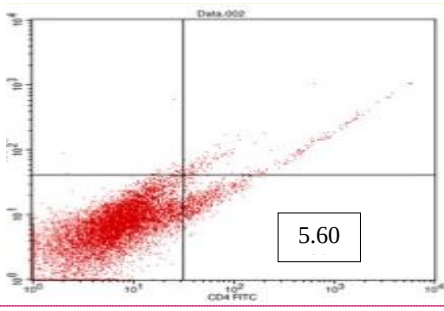

Figure 2. T lymphocytes (CD4 +) cells expression of (5.60%) in the sali~~v~~a of ~~Free~~ Caries Free

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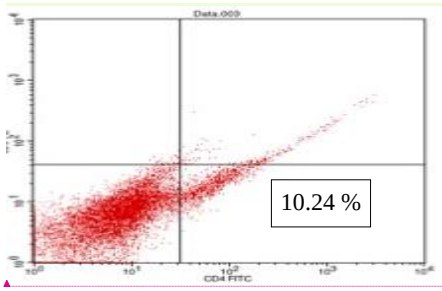

Figure 3. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

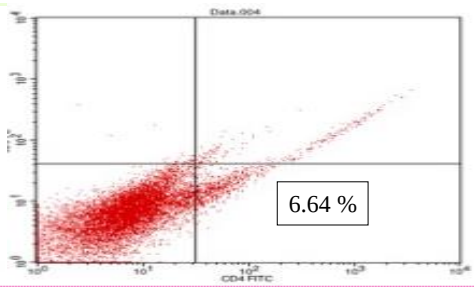

Figure 4. T lymphocytes (CD4 +) cells Expression (6.64%) in the saliva of S-ECC ~~salivary~~

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

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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 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.

6b. 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.

7c. 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. 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. 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. Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen SGet al. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. J Virol. 2014; Sep;88(17):9934–46.

11. Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AKet 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 2016;113(20): 5682–5687.

14. Luthfi M, Setijanto D, Rahardjo MB, Indrawati R, Rachmadi P, Ruth MSet 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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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]

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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.

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interactions. *Proc Natl Acad Sci U S A.* (2016) 113:5682–7. doi: 10.1073/pnas.1513918113 10.108413513918113 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

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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

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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

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

Letter of Abstract Acceptance

2 pesan

INSBIOMM Scientific Commitee <insbiomm@gmail.com> 13 Agustus 2019 15.45
Kepada: m.luthfi@fkg.unair.ac.id

To: **Mr Muhammad Luthfi**

INSBIOMM
International conference on latest perspectives on Infectious Diseases, including Biotreats and Military Medicine
August 27-28th, 2019, Surabaya, INDONESIA

Letter of Abstract Acceptance

Dear Presenter,

We are very pleased to inform you that your abstract entitled, **"Difference of Expression Of Limfosit T (Cd4 +) Cells In Severe Early Childhood Caries And Free Carie "** has been **accepted** for **Poster** presentation at International conference on latest perspectives on Infectious Diseases, including Biotreats and Military Medicine (INSBIOMM) scheduled on August 27-28, 2019 in Surabaya, Indonesia. The exact time and room of your presentation session will be specified on the INSBIOMM website: http://itd.unair.ac.id/insbiomm/ at the beginning of August, 2019.

Please note that individual requests for specific presentation dates and/or times cannot be addressed. Oral presentations can not exceed 10 min (including disscussion). The details of oral presentation guideline is available on the conference website.

It is a condition of abstract acceptance that you or a nominated presenting co-author completes the registration and payment process before August 19th, 2019. Registration to attend the conference, please follow the link: http://itd.unair.ac.id/insbiomm/ Should the addressee above not be the nominated presenter, please inform us the name and email address of the presenter immediately to: insbiomm@itd.unair.ac.id; insbiomm@gmail.com

Again, congratulations on the acceptance of your abstract. If you are interest to publish your full paper to our proceeding or journal of **"Difference of Expression Of Limfosit T (Cd4 +) Cells In Severe Early Childhood Caries And Free Carie "** please submit your full paper to : insbiomm@itd.unair.ac.id; insbiomm@gmail.com On behalf of the Scientific Program Committee, we look forward to your full participation in the INSBIOMM 2019 in Surabaya.

Yours Sincerely,

Prof. Soetjipto, dr., MS., Ph.D.
Chairman of the Organizing Committee
Institute of Tropical Disease, Universitas Airlangga
Kampus C UNAIR
Jl. Mulyorejo, Surabaya
Zip code: 60115
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MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> 13 Agustus 2019 20.29
Kepada: INSBIOMM Scientific Commitee <insbiomm@gmail.com>

We have sent 2 manuscripts full paper and proof of transfer

best regards
luthfi

Pada tanggal Sel, 13 Agu 2019 pukul 15.45 INSBIOMM Scientific Commitee <insbiomm@gmail.com> menulis:

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Request of Revised

INSBIOMM Conference <insbiomm@gmail.com>
Kepada: MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

8 Januari 2020 04.03

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.

Please revised this manuscript as peer the comment.

The revised itself is due : 2019-01-13

Best Regards
Scientific Committee

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International Conference on
Infectious Diseases, Biothreats, and Military Medicine
INSBIOMM 2019
* Secretariate:
Institute of Tropical Disease
Kampus C Unair, Jl. Mulyorejo, Surabaya 60115
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e-Mail: insbiomm@itd.unair.ac.id, insbiomm@gmail.com
Phone/WhatsApp: +6281325267661

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