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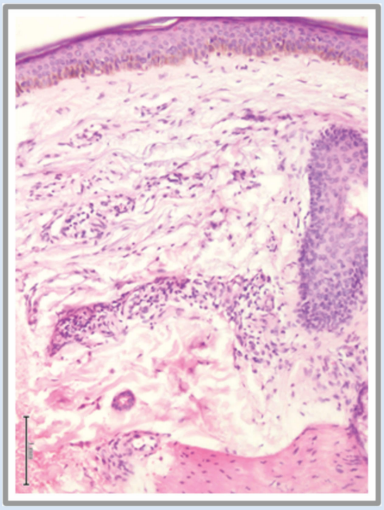
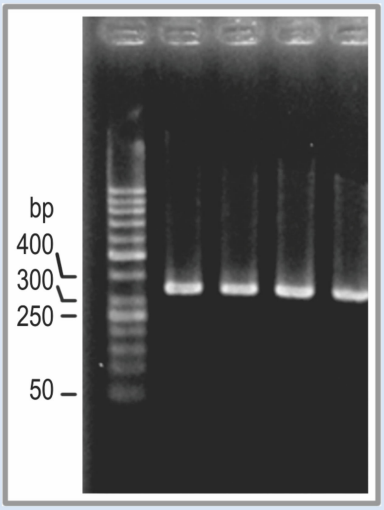
PROCEEDING

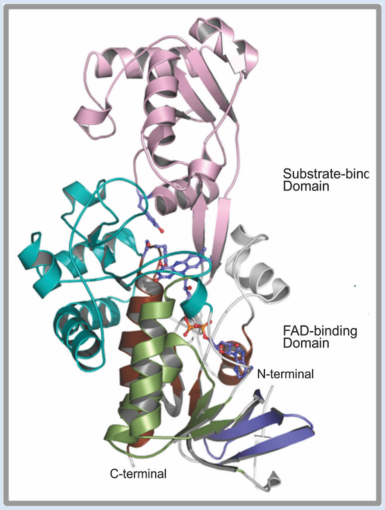
Institute of Tropical Disease
Universitas Airlangga

Institute for Protein Research
Osaka University

Molecular and Cellular Life Sciences:
Infectious Diseases, Biochemistry and Structural Biology







Editors:

Prof. Dr. Ni Nyoman Tri Puspaningsih
Prof. Dr. Maria Inge Lusida
Dr. Juniastuti
Dr. Mirny Lamid
Dr. Purkan
Ali Rohman

Molecular and Cellular Life Sciences (MCLS) Conference
Surabaya, 7 – 8 May 2015

Proceeding

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Infectious Diseases, Biochemistry and Structural Biology

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Preface

The International Seminar on Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry & Structural Biology (MCLS 2015) was held in Hotel Pullman Surabaya City Centre, Surabaya, Indonesia, on 7 – 8 May 2015. This seminar was organized by the Institute of Tropical Disease, Universitas Airlangga, Indonesia in a very productive collaboration with the Institute for Protein Research, Osaka University, Japan. The seminar program included plenary lectures, invited lectures, oral presentations, poster exhibition, as well as a welcome visit to the house of the Mayor of Surabaya. In total of 208 scientific participants, 14 of whom are invited speakers, contributed to this conference. During conference, they had very effective shares and discussions in the fields of infectious diseases, biochemistry, and structural biology. MCLS 2015 was indeed in a truly international atmosphere. The participants came from 9 different countries, *i.e.* Australia, Indonesia, Japan, Malaysia, Netherlands, Singapore, Thailand, Taiwan, and Vietnam.

In order to spread the seminar outcomes, I would like to introduce you a conference proceeding (Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology, ISBN 978-602-14292-4-2. A total of 62 papers were submitted to MCLS 2015 and each paper was reviewed by three peers. Of these reviewed papers, 32% were selected to be published in this proceeding. My grateful thanks to you all the peer-reviewers, who are Prof. Toshiharu Hase (Osaka University, Japan), Prof. Bauke W. Dijkstra (University of Groningen, Netherlands), Prof. Nicholas E. Dixon (University of Wollongong, Australia), Prof. Genji Kurisu (Osaka University, Japan), Prof. Ni Nyoman Tri Puspaningsih (Universitas Airlangga, Indonesia), Prof. Maria Inge Lusida (Universitas Airlangga, Indonesia), Dr. Juniastuti (Universitas Airlangga, Indonesia), Dr. Mirny Lamid (Universitas Airlangga, Indonesia), Dr. Purkan (Universitas Airlangga, Indonesia), and Ali Rohman (Universitas Airlangga, Indonesia).

On behalf of the organizing committee, in this joyful moment I would like to express my sincere gratitude to Prof. Fasich (Rector of Universitas Airlangga), Prof. Saburo Aimoto (Executive Vice President of Osaka University), Prof. Dr. Nasronudin (Director of the Institute of Tropical Disease, Universitas Airlangga), Prof. Haruki Nakamura (Director of the Institute for Protein Research, Osaka University), and Prof. Toshiharu Hase (the Institute for Protein Research, Osaka University) who facilitated this event to be smoothly taken place. I would also like to thank all the invited speakers for their discussions and sharing. They are Prof. Toshiharu Hase (Osaka University, Japan), Prof. Bauke W. Dijkstra (University of Groningen, Netherlands), Prof. Nicholas E. Dixon (University of Wollongong, Australia), Prof. Genji Kurisu (Osaka University, Japan), Prof. James R. Ketudat Cairns (Suranaree University of Technology, Thailand), Prof. Chun-Jung Chen (National Synchrotron Radiation Research Center, Taiwan), Prof. Bambang Sugiharto (Jember University, Indonesia), Prof. Kiyoshi Kita (University of Tokyo, Japan), Prof. Eiji Konishi (Mahidol University, Thailand and Osaka University, Japan), Prof. Atsushi Nakagawa (Osaka University, Japan), Prof. Robert C. Robinson (The Agency for Science, Technology and Research (A*STAR), Singapore), Prof. Hitoshi Sakakibara (Nagoya University, Japan), Prof. Ni Nyoman Tri Puspaningsih (Universitas Airlangga, Indonesia), and Prof. Maria Inge Lusida (Universitas Airlangga, Indonesia). Moreover, I wish to convey my sincere appreciation to the Mayor of Surabaya Madam Dr. (H.C.) Tri Rismaharini for inviting our distinguished guests to a warm welcome dinner in her official residence. An event such this conference requires a lot of work from many people. Therefore, I take this opportunity to thank to the Organizing Committee of MCLS 2015 and all people who supported this conference in various ways.

Last but not least, we hope that this proceeding provides a valuable contribution for the development of science and technology, especially in the area of infectious diseases, biochemistry, and structural biology.

Sincerely yours,

Maria Inge Lusida
Chairperson of the Organizing Committee

Scientific Committee of MCLS 2015

in a collaboration with

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Analyze Activation Marker of Azurophilic Granule (CD63) and CFSE CD11C Expressions of Salivary Neutrophils Insevere Early Chilhood Caries

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Abstract

Early childhood cariesis is a very serious health problem because it is a chronic infectious disease that is infectious. In recent years the view has changed dramatically neutrophils, where neutrophils which are a key component of the first line of defense against pathogen through the process of phagocytosis. Neutrophils also release their granule contents such as defensins, Defensins (α defensins) are stored in the azurophilic granules have microbicidal function shown in enable macrophages to release tumor necrosis factor (TNF) and interferon γ (IFNγ) which promote pro inflammatory. α Defensin also increases the permeability of the epithelial monolayer in vitro and chemotactic effects on T cells, mast cells and dendritic cells. Purpose this research is to analyze activation marker of azhurophilic granules and CFSE CD11c expression of salivary neutrophils in severe early childhood caries (S-ECC). Two groups ie, results mouthwash NaCl 1.5% samples of 20 early childhood caries-free and 20 severe early childhood caries. Salivary neutrophils that collected from severe early childhood caries analyzed using flow cytometry to detect the CD63 and CFSE CD11C expressions. Based on the average value is known that salivary neutrophils expressing $CD63^+$ in early childhood caries-free higher (2.67% ± 0.46) in comparison to the severe early childhood caries (2.32% ± 0.57), likewise salivary neutrophils expressing $CFSECD11c^+$ in early childhood caries-free higher (2.44% ± 0.52) in comparison to the severe early childhood caries (1.57 ± 0.39). Decreased activation marker azhurophilic granules (CD63) and phagocytosis markers (CFSE CD11c) expressions may be one cause in the S-ECC.

Keywords: severe early chilhood caries; neutrophils; azhurophilic granules; phagocytosis.

1. Introduction

Early childhood caries is a very serious health problem. In 2003 the American Academy of Pediatric Dentistry (AAPD) has declared severity of the disease because it is a chronic infectious disease that menular[1]. Dental caries begins after deciduous teeth grow and develop on the surface of the tooth with a very fast and progressive with manifestations of pain, acute and chronic abscesses, fever, swelling of the lips so that the appetite decreased[2].

Severe early childhood caries with decay exfoliation filling of teeth (def-t) > 6 is a form of very destructive because it involves several teeth, including maxillary anterior teeth[3], with the signs is the smooth surface decay on children under three years of age and usually begins as soon as the first tooth eruption and growing rapidly to cavitation stage performance only 6 to12 months[4].

Early childhood caries prevalence is still very high, the results showed that the prevalence reached 80% in developing countries with a percentage five times more than asthma, seven times more than allergies, and fourteen times more than chronic bronchitis[2,5].

In Indonesia, Fitriani research results in 2007 in Semarang, show that at 90.5% in urban and 95.9% rural early childhood suffered from dental caries, while in Surabaya in 2006, the results of research conducted Indawati showed that 74% early childhood suffered from dental caries[6].

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Dental caries is a chronic disease, is multi factorial which began with the shift of microbiology in the complex biofilm (dental plaque) were affected by the consumption of sugar, salivary flow, and behavior[7,8]. The shift toward bacterial plaque flora acidogenic and aciduric in a long time so that the pH of the plaque to be low which is accompanied with many foods containing sucrose and the factors involving oral hygiene, the aging, genetic factors, and changes in the immune system, making conditions the plaque that support the increasing number of species acidogenic and acid uric like *Streptococcus mutans* (*S.mutans*) or laktobasilus[9].

Untreated dental caries can cause more severe effects on the body, including mortality[10]. The poor nutritional intake associated with pain in early childhood can reduce proper diet so that the immune system weakens and becomes more susceptible to infection. Various prevention of dental caries has been done, for example by brush their teeth, fluoridation by topical application and manufacture of vaccines to date have not shown the expected results[11].

In recent years the view has changed dramatically neutrophils, where neutrophils which are a key component of first line of defense against mikroba[12]. Neutrophil not only act as the killing of microbes by phagocytosis, release of reactive oxygen species (ROS) and its antimicrobial peptide but also regulate activation of neutrophils immune responses[13], besides neutrophils has resulted in a variety of cytokines, chemokines and growth factors that become a major contributor in the production pro inflammatory cytokines in the area of infection[14].

Important function of neutrophils in killing pathogenic microbes is phagocytosis, which was significantly more effective due process of opsonization by antibody and complement contained in the surface of microbes. Phagocytosis against microbes can causes oxidative burst process of producing reactive oxygen species are accompanied by degranulation of cytoplasmic granules in the phagosome containing microbes that contains antimicrobial peptides and protease[15].

During phagocytosis, there was internalization of microbes into the phagosome in neutrophils. Receptors involved in the recognition of microbes also activate neutrophils to kill microbes are ingested. A combination of the phagosome with neutrophil granules (lysosomes) leads to the formation of phagolysosome and at this point most of the microbicidal activity[16].

Neutrophil contains three types of primary granules, granules azurophilic (primary granules) contain antimicrobial proteins such asdefensins (HNP 1-3), elastase, cathepsinand proteinase-3 and contains CD63 highly potent in killing microbes both Gram positive and Gram negative, including *S.mutans* which is one of the predominant microbial cause of dental caries.

Based on the above, this study aims to identify risk factors for caries of the aspects of the immune system to identify neutrophil function as innate immunity effect or cells in preventing dental caries in early childhood.

2. Methods

Samples were obtained from kindergarten children in Surabaya region. Examination of dental caries was conducted by measuring its index of def-t, and after it has been examined subjects were divided into two groups: caries-free group and the S-ECC group with def-t more than 6. All subjects at the time of sampling old between 4 to 6 years. Prior to sampling in a sample, the distribution of questionnaire and signed a written informed consent by parents respectively.

Samples of saliva were taken to the isolation of *S.mutans* and from the mouth wash NCL 1.5% for the determination of phagocytic function analyzed by CFSE expression of CD11c and CD63 expression is a marker granulation azurophilic granules on the surface of neutrophils saliva. Sampling was done by researchers and trained person elusing a standard protocol. Subjects should not eat, drink, chew gum, or brushing teeth for 60 minutes before sampling. Having collected the samples was frozen at -80°C for analysis[17].

2.1. Streptococcus mutans isolation from saliva

Isolation of *S.mutans* conducted from saliva samples taken from both preschool children identified as severe caries and caries-free conducted in the following way: isolation and biochemical characterization of *S.mutans* saliva samples were diluted in Brain Heart Broth (BHI), after incubation 24 hours.

Samples were grown on Triptone Yeast Cysteine (TYC) agar medium. Suspected colonies of *S. mutans* maked subculture to conducted biochemical tests using fermented mannitol, raffinose, sorbitol, salicin, esculin and arginine. Isolates were identified as *S. mutans* if positive for sugar fermentation and negative for arginine and subsequently confirmed by Gram staining and catalase test. Isolates of *S. mutans* is stored on -80°C[17].

2.2. Isolation and Counting Total Salivary Neutrophils

Neutrophils in saliva obtained by subjects instructed to rinse with 10 mL of sterile 1.5% NaCl solution as he gargled his mouth but not swallowed for 30 seconds, then expectorationed in sterile glass. This procedure was repeated 4 times. The next sample is centrifuged at a speed of 450 g for 15 minutes, at a temperature of 4℃. The results of further centrifugation pellets are mixed with 2 ml of RPMI medium then the sample in vortex then

filtered sequentially with 20 and 11µm nylon filter19. The results of the filter in the form of a suspension of cells is then counted using a hemocytometer.

2.3. Preparation of the Streptococcus mutans (S. mutans)

S. mutans isolated subsequent cultured in media Brain Heart Infusion (BHI) so that the tube for 48 hours in an aerobic jar. *S. mutans* culture results subsequent taken 1 ose and cultured back on BHI liquid medium for 48 hours in an incubator. S. *mutans* bacteria cultures are then put into the microtube and centrifuged at 12,000 rpm for 10 minutes at a 4℃. Pellets were then washed with PBS and centrifuged again with the speed, time, and temperature are the same. Pellets were then stained with 50 µl solution CFSE: PBS in the ratio 1:20. Bacteria that have dyed subsequent incubated in a 37℃ temperature for 30-60 minutes in the dark (microtube coated aluminum foil). After incubation, the bacteria then centrifuged at a speed of 12,000 rpm, for 10 minutes, at a temperature of 4℃. Pellets were then washed with PBS 2 times by means of centrifugation back at the same speed. *S. mutans* bacteria were then killed by heating 60℃ for 30-60 minutes. The bacteria were then washed with PBS and measured at 620 nm (0.35). Bacteria opsonisationed subsequent use as much 500 µl serum FCS and incubated at 37℃ for 30 minutes. The next steps are conducted centrifugation with the same speed as before. Pellets containing bacteria, calculated using the haemocytometer to be taken and planted in the method of phagocytosis.

2.4. Phagocytosis activity Salivary Neutrophils against *S. mutans*

The principle underlying the process of phagocytosisis caused by bacteria can be labeled with fluorescein isothiocyanate (FITC) and then bacteria difagosit by neutrophils, after it was given a second dye in the form of ethidium bromide (EB) which serves to bind DNA. EB is what will give color to the *S. mutans* is not in phagocytosis because the microbes that have internalized protected by a membrane of phagocytesthat is not exposed to the dye. At Flowcytometer light scattering can be used to isolate a population of neutrophils. The combination of fluorescence and dissemination will identify *S. mutans* that has been labeled, neutrophils, and neutrophil containing *S. mutans* is ingested.

Neutrophils that have been isolated and regulated concentration calculated to be 2 X IO^6/ml. Make sure that the cells are predominantly neutrophils (>95%) and the life (>95%) after the test with trypan blue propidium iodide by flow cytometer, thus cells are now ready to test phagocytosis.

In the phagocytosis test, the addition of 5 ml suspension as much as 2 X IO^6 neutrophils/ml of bacteria to the tube. Then 1 ml was transferred into a tube containing 1 ml of ice cold, 0.9% and 0.02% EDTA saline as a control. The same procedure was repeated every 15 minutes with new aliquot. This will give the results of phagocytosis over a period of 1 hour.

The engulfed of bacteria is determined by measuring green fluorescence at 525 nm in flow cytometry, using 488 nm excitation. To estimate the extra cellular fluorescence, immediately after running each tube add 1 ml of 3 mg/ml trypan blue, and then mixed and measured fluorescence signal again.

2.5. Measurement of CD63 Expression On Salivary Neutrophils

Profile measurement of neutrophil cells using CD63 antibody which is a marker of neutrophil activation. CD63 is the primary granule membranes were expressed on the membrane surface of neutrophils and increased due to the stimulation of the neutrophil cells (Faurschou and Borregaard, 2003) with the signs that can be measured by flow cytometry with the method that has been modified Bjornsson11. The sample used is a sample result of 1.5% NaCl mouth wash that has been isolated neutrophils its. Neutrophil cell suspension that was isolated and then inserted into the microtube that has been filled as much 500 µl PBS. The cell suspension was then centrifuged at a speed of 2500 rpm, for 5 minutes at a temperature of 4℃. Pellets obtained were then stained with antibody extracellular much 50 µl (Biolegend antihumanα-CD63PE), and α-PI Biolegend PE conjugated antibody ratio : PBS is 1: 200.

Cells that have been added antibody is then stored at 4°C for 30 minutes. Then the cell suspension added 1 ml PBS and centrifuged at 2500 rpm, for 5 minutes at 4°C. Pellets then added Biolegend Cytofix Cytoperm as 100 µl and homogenized until well blended. Do incubation in the dark and 4°C for 20 minutes. After incubation, cells were then added 1 mL Biolegend Washperm 1X and then centrifuged at a speed of 2500 rpm, for 5 min at 4°C. Pellets were then added intracellular antibodies, including BD antihuman α-CD64 PerCP conjugated, subsequentin corporated the cell suspension into flow cytometer cuvette, added as much as 300 µl PBS, and mounted on then ozzle to conducted BDFACS Calibur flow cytometer using the machine running. Samples were analyzed by flowcytometry (FACS Calibur flow cytometer, BD BioSciences, SanJose, CA).

Neutrophil gate identified by using density and size with aside angle light scatter and then forwarded to the forward angle light scatter. This compensation is achieved by using FITC and PE antibodies dilebel with the individual. The results are expressed as mean fluorescence intensity (MFI). FACS Calibur from Becton

Dickinson Cell Quests of ware program used for the analysis.

3. Results and discussion

3.1. CFSECD11C expression In Salivary Neutrophils at S-ECC and Free Caries

The test results of two independent samples T test showed that there was a significant decrease in the expression of CFSECD11c in salivary neutrophils S-ECC shown in Table1.

Table 1. The mean and standard deviation salivary neutrophils activated conduct phagocytosis of *S. mutans* were labelled by CFSE dye express $CD11c^+$ in S-ECC and caries-free (%)

Group	n	Mean± standard deviation standard deviation	SD	P Value
Free Caries	20	2.44% ± 0.5	0.52732	0.000 (p<α)
S-ECC	20	1.57 ± 0.39	0.39038	

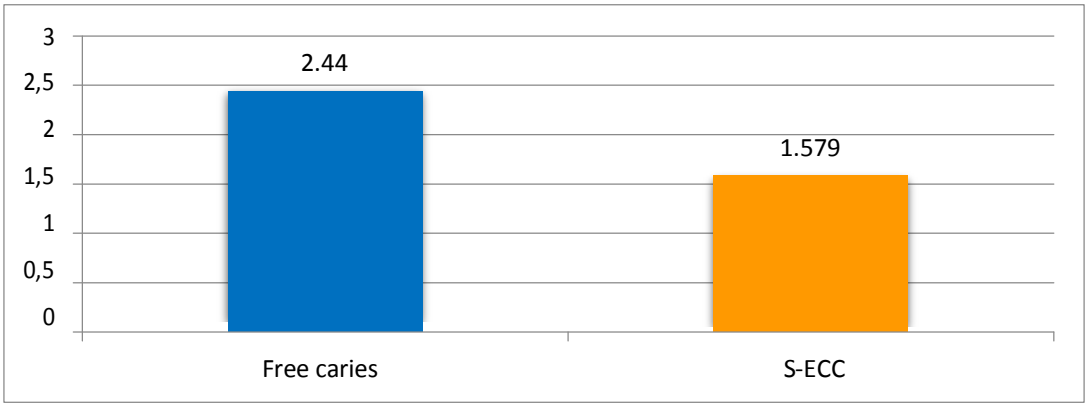

Fig.1. Phagocytosis of bacteria *S. mutans* were labeled by CFSE dye express $CD11c^+$ in S-ECC and free caries (%).

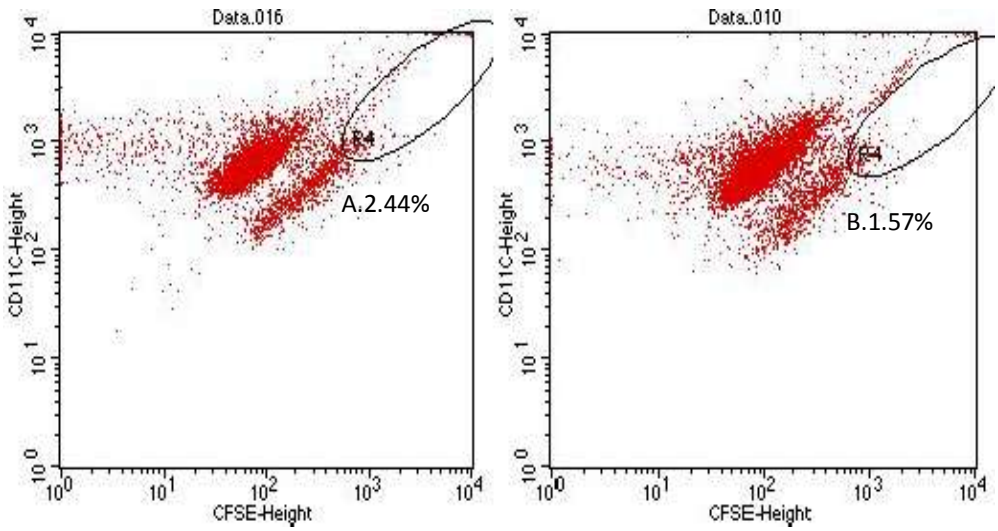

Fig.2. Activated salivary neutrophils conduct phagocytosis against *S. mutans* which in CFSE label edexpressing $CD11c^+$ were detected using flow cytometry on early chihood caries-free (A) and S-ECC (B)

3.2. $CD64^+CD63^+$ Expression On Salivary Neutrophilsin S-ECC and Free Caries

Results of the analysis of activated salivary neutrophils ($CD64^+$) that expresses $CD63^+$ in early Childhood free caries and S-ECC are shown in Table 2.

Table 2. The mean and standard deviation of activated salivary neutrophils ($CD64^+$) that expresses $CD63^+$ in early Childhood free caries and S-ECC (%)

Group	N	Mean± Standard deviation	95%CI	p Value
Free Karies	20	2.67 ± 0.46	2.37–2.96	0.040 ($p<\alpha$)
S-ECC	20	2.32 ± 0.57	1.96–2.68	

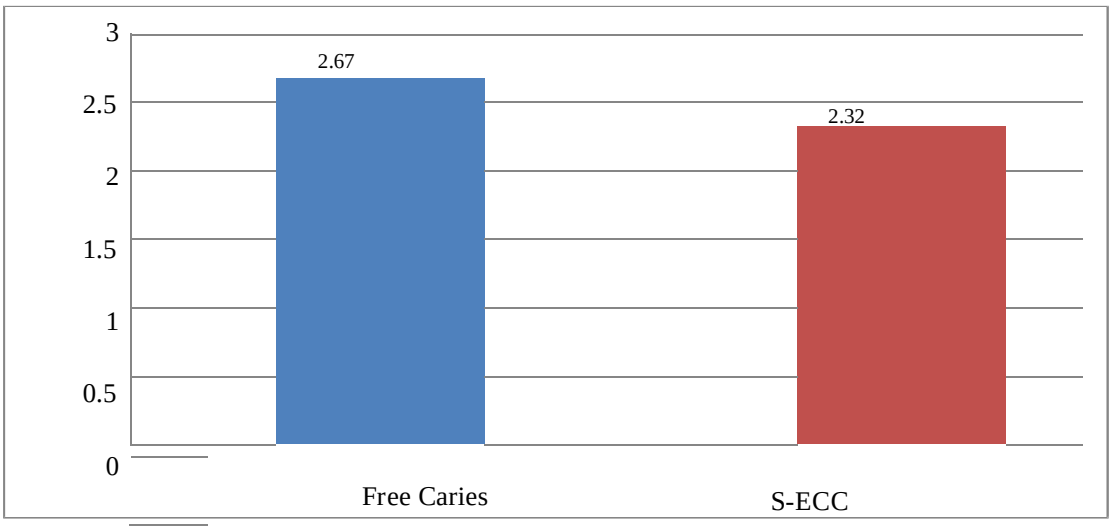

Fig.3. The mean and standard deviation saliva activated neutrophils ($CD64^+$) that expresses $CD63^+$ in early childhood caries-free and S-ECC (%)

The results of flow cytometry analysis using saliva activated neutrophils ($CD64^+$) that expresses $CD63^+$ which has been done using a different test independent 2 samplest-test showed significant value which is smaller than α, this means that there are significant differences in the exp ression of $CD63^+$ between the two groups. Based on the average value of salivary neutrophilsis was known that expressing $CD63^+$ in early childhood caries-free higher (2.67% ± 0.46) compared in S-ECC (2.32% ± 0.57).

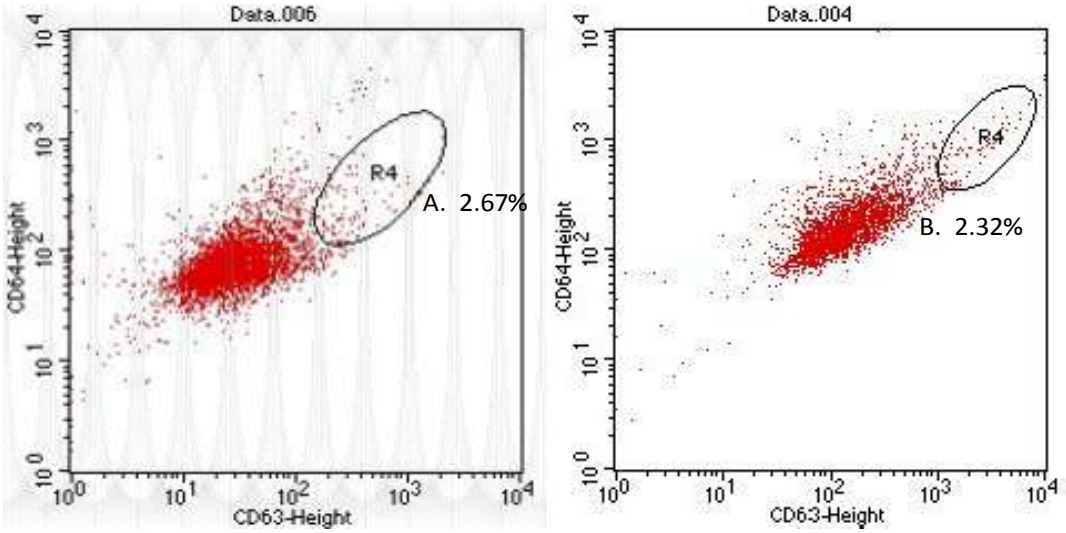

Fig.4. Salivary Neutrophil activated ($CD64^+$) expressing $CD63^+$were detected using flow cytometry in early childhood free caries (A) and in S-ECC (B)

Streptococcus mutans plays an integral role etiology of dental caries in preschool children is an infectious disease transmitted[18], so that *S. mutans* is considered an important predictor as cariogenic bacteria because it is acidogenic (able to produce acid) and acid uric (able to survive in an acidic environment)[3].

In salivary neutrophil first line of defense is the most prominent of immune cells for defense against pathogens microbial. The importance of neutrophils in the host immune system in patients with neutropenia or deffects in neutrophil function which leads to a tendency for the occurrence of serious infections[19].

Neutrophil recruitment process, transmigration, phagocytosis, and activation of highly coordinated to prevent or eliminate infection in humans. In the area of infection, neutrophils bind and engulf microbes through a process known as phagocytosis. Neutrophils recognize the surface-bound or free molecules secreted bacteria, including peptidoglycan, lipoprotein, lipoteichoic acid (LTA), lipopolysaccharide (LPS), CpG-containing DNA, and flagellin. This pathogen molecules known as PAMPs, interact directly with a number of Pathogen Recognition Receptors (PRRS) expressed on the surface of cells, including Toll Like Receptors (TLRs)[20].

Dental caries severity varies greatly between individuals, and this variability may be due to differences in the microflora as well as differences in the immune system in response to oral microflora in general, the immune system in the oral cavity to prevent the invasion of the microflora[21].

Neutrophils are important effect or cells in the first line of defense against pathogenic bacteria through the process fagositosis[22], the disruption phagocytic function against cariogenic bacteria may play an important role in the initiation and progress ion of dental caries. This, our study shows that the percentage of neutrophil phagocytosis against *S. mutans* which is the bacteria that cause dental caries showed significant declines in the S-ECC than in early childhood caries-free.

The ability of the pathogen to avoid internalizing and killings played a central role in the virulence strategies. Pathogens that have been internalized through a process of phagocytosis by neutrophils to developing three strategic defense system to avoid killing intracellularly is the first escape from the phagosome, the second blocking phagosome fusion-lisosome, and the third is the use of a mechanism to allow for the survival of the phagolysosomes[22]. Some species of bacteria including Streptococcus and Staphylococcus have evolved mechanisms to avoid opsonophagocytosis or action of the system complemen[23]. The failure of the existing defense systems in saliva in early childhood in killing the *S. mutans* bacteria due to the possibility that there are strains of *S. mutans* in the oral cavity S-ECC has successfully developed a mechanism to fight the hydrolytic activity of lysozyme. This is supported by studies that say that some Streptococcus have an effective strategy to reduce the bactericidal effect of lysozyme.

Phagocytosis is active receptor-mediated process, in which cells internalize microbial later there cytoskeletal rearrangements, neutrophil plasma membrane extends around the target, initiate a process that ultimately creates a membrane-bound vacuoles called the phagosome. Neutrophil release granule mediators that are released upon degranulation orexocytosis of membrane-bound secretory granules. Neutrophils also have the capacity to release a variety of antimicrobial proteins and intracellular enzymes into membrane-bound organelle called the phagosome containing microbes.

Primary granules (azurophilic) contains many antimicrobial compounds such as myeloperoxidase (MPO), defensin in the form of human neutrophil peptides1-3 (HNP1-3), lysozyme, azurocidin, and serine proteinase elastase, cathepsin G, proteinase 3, esterase N, and so on. These granules associated with phagocytic vesicles release their contents in the phagosome causing microbes that have in phagocytosis[24].

The low expression of CD63 neutrophil saliva in severe early childhood caries may be due to *S. mutans* that has been internalized by phagocytosis of neutrophils through a process that is mediated through FcαR (CD89) or CR1 (CD35) might be able to develop three strategic defense system to avoid killing intracellular namely that the first escape phagosome, the second blocking phagosome fusion-lisosome, and the third is the use of a mechanism to allow for the survival of the phagolysosomes[22].

Phagocytosis by neutrophils causes translocation of granulesto the phagosome which is a marker for the release of the granules contents. Caused by the release of granule contents as the mediator of granulocytes whicha re strictly controlled by a mechanism leading to exocytosis which took place in several phase[25], namely the recruitment of granule exocytosis from the cytoplasm to the membrane targets depend on rearrangement of actin and microtubulecy to skeleton followed by vesiclete the ring and docking, which leads to contact the outer surface of the lipid bilayer membrane around the granules with the inner surface of the target membrane[26].

The role of neutrophils as innate immunity lies in the lysosomal compartment shown in congenital abnormalities which cause the secretion of lysosomal events and lysosomal proteolysis disrupted as happens in Chediak-Higashi[29] Syndrome. And Lefevre Papillon-syndrome[28]. The function of lysosome-associated membrane protein-2(-2LAMP) contained in neutrophil function is critical to the self-cleaning of the oral cavity that regulates natural defense against biofilm formation in the oral cavity. Phagosome fusion-lisosome is very important for the degradation of the internalized pathogens efficiently with lysosomes thus play an important role in the killing of bacteria that do not depend on oxygen[29]. Neutrophil-deficient LAMP-2 caused deficiency of distribution and azurophilic granule fusion, cellular localization lactoferrin contained in the secondary granules which showed a reduction inco-localization of causing disruption of biogenesis, traffic, both types of granulesis a function that contributes to killings *S. mutans*[30] and it is a possibility that lysosome-associated membrane protein-2(LAMP-2) to contribute to fusion with neutrophil granules in phagosomes. Based on the low expression of CD63S-ECC possibility of deficiency LAMP-2 as found in mice that were suffering from periodontitis therefore LAMP-2 deficiency.

4. Conclusion

Decreased activation marker azhurophilic granules (CD63) and phagocytosis markers (CFSE CD11c) expressions may be one cause in S-ECC.

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