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Original Research

Analysis of Interleukin-10 Anti-inflammatory Cytokines in Salivary Lymphocyte Surface: A Pilot Study

Retno Indrawati[1], Muhammad Luthfi[1], Aqsa S. Oki[1], Yuliati[1], Agung Sosiawan[2], Priyawan Rachmadi[3], Muhaimin Rifai[4]

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Abstract

Aim: The aim of this study was to analyze the expression of interleukin-10 (IL-10) in children with severe early childhood caries (S-ECC) and caries-free children. **Materials and Methods:** This was an observational analytic pilot study performed on children with social factors-ECC (S-ECC), and caries-free children as the objects of research with a cross-sectional study design. Saliva of children aged 4–6 years from the group of caries children in severe and caries-free early childhood was taken. Samples were taken by rinsing with 1.5% sterile NaCl for 30 s and then accommodated in a sterile tube, to get a 40 mL sample from the aforementioned procedure repeated four times. Flow-cytometry test was used to analyze the IL-10 expression. The results of the study were analyzed using the normality test using Shapiro–Wilk, then continued with *t* test using the Statistical Package for the Social Sciences (SPSS) software program, version 20.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by independent *t* test to see the difference between caries-free children and S-ECC. **Results:** The expression of IL-10 in the saliva of children with severe ECC was 3.32 ± 0.79; meanwhile, in caries-free children it was 4.04 ± 0.65. **Conclusion:** The IL-10 expression in children with severe ECC was significantly lower than that of in caries-free children.

Keywords: Interleukin-10 Anti-inflammatory Cytokines, Lymphocyte Cells, Severe Early Childhood Caries

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Introduction

Dental caries is a multifactorial disease due to various factors, namely cariogenic microbes, carbohydrates, and social factors, whereas early childhood caries (ECC) is often found in children with low-socioeconomic conditions.[1] Several studies have recognized the importance of infection of *Streptococci mutans*.[2]

The immune system is a very varied compilation of cells, consisting of two parts of the immune system, namely innate and adaptive. The innate and adaptive immune systems are interrelated, and recognition by innate immune systems can cause the activation of the adaptive immune response.[3] The innate immune system is the first line of host defense against pathogens and recognizes molecules repeatedly against pathogens, which are called pathogen-related molecular patterns through germline-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[4]

The components that regulate the immune system, such as an immune regulator cells and regulating cytokines, both natural and acquired as induced by an antigen, plays an important role in controlling various immune responses, both physiological and pathological. Local and systemic interleukin-10 (IL-10) responses have been shown to have pathophysiological relevance in several diseases such as malignancy, infectious diseases, autoimmune diseases, and atopic disorders.[5] Because of

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this, IL-10 can activate signal transducer and activator of transcription 3 (STAT3) in macrophages and T cells to restore and respond to the presence of pro-inflammatory cytokines.[6]

Immunity in the oral cavity's immune system has an important role that is balancing the amount of microbes in the mouth. The microbial activity in oral cavity can be fluctuatives, due to pathogen situations. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system. The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine, which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines that function to limit tissue damage and to maintain tissue homeostasis.[7] IL-10 is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

For dental caries preventions, many efforts had been carries out. The government and supporting health organization ran some prevention programs, such as dental counseling to community. Most of the programs targeted children, pregnant woman, and elderly. They teach how to brush teeth properly, dietary that good for dental health, prevention treatment that can be applied for children, and vaccines.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of social factors-ECC (S-ECC).

Materials and Methods

This was an observational analytic study using children with S-ECC and caries-free children as the objects of research with a cross-sectional study design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 209/HRECC.FODM/IX/2017).

Sixteen children with S-ECC and caries-free were taken from preschool children aged 4–6 years, in the southern Surabaya region, which had previously been divided into two groups.

Group one were children with a diagnosis of S-ECC characterized by decay, extraction, and filling (def-t > 6), whereas, the second group were preschool children who were diagnosed with free caries marked with def-t = 0.

5 mL saliva is taken from preschool children with S-ECC and caries free. Sampling was carried out by researchers and trained research assistants using standard protocols. Subjects were asked not to consume food and drink, or brush their teeth for 60 min before the study was conducted. The samples obtained were stored at −80°C for analysis. IL-10 expression was analyzed using flow cytometry, according to Luthfi *et al.*[10]

Statistical analysis: The data were analyzed by independent t test to see the difference between caries free and S-ECC.

Results

Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the SPSS Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

The data obtained indicate that the average IL-10 in the caries-free group was higher than the S-ECC group, but the difference was not statistically significant between caries-free and S-ECC.

Discussion

From the data obtained from the research results then performed statistical calculations. Before testing and analysis between S-ECC groups and caries free, the normality test was done in each group using the Shapiro-Wilk test which obtained the result that the value of $p > \alpha = 0.05$ is $P = 0.844$ which means that the data are normally distributed, as shown in table 1 and in table 2 is the result of statistical analysis between S-ECC and caries free using independent t test, the value of $p = 0.11$ means that there are differences in expression even though statistically it is not showed significant results because the value of

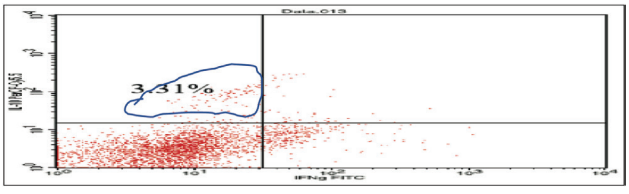

Figure 1: Expression of interleukin-10 (3.31%) from severe early childhood caries salivary after analyzed by flow-cytometry test

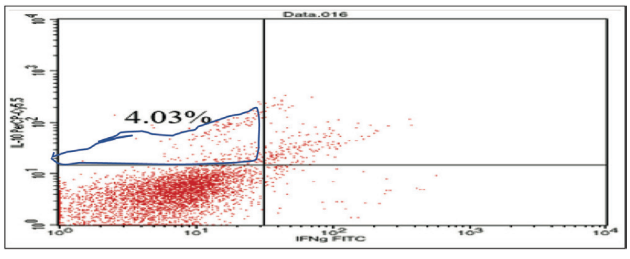

Figure 2: Expression of interleukin-10 (4.03%) from caries free salivary after analyzed by flow-cytometry test

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Table 1: Normality test using Shapiro–Wilk interleukin-10 expression from severe early childhood caries and caries free

Variable	Kolmogorov–Smirnov			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
	143	16	200	970	16	844

IL-10 = interleukin-10, Df = degrees of freedom

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Table 2: Mean and standard deviation of interleukin-10 expression in severe early childhood caries and caries free analyzed by flow-cytometry test, which was tested using independent *t* test

Group	*n*	IL-10 expression (%)	
		Mean ± SD	*P*
Caries free	8	4.04 ± 0.89	0.11
S-ECC	8	3.32 ± 0.76	

S-ECC = severe early childhood caries, SD = standard deviation, IL-10 = interleukin-10

p> 0.05. on the surface of lymphocyte cells in saliva the S-ECC group that expressed IL-10 (3.31%) was less than that of the caries free group that expressed IL-10 (4.03%) this will be clarified in Figures 1 and 2 .

Based on Figure 1 which is the result of examination using flow cytometry test shows that lymphocytes in saliva severe early childhood caries express IL-10 of 3.31%, while in Figure 2 which is the result of examination using flow cytometry test shows that lymphocytes in caries-free children express IL- 10 at 4.03%. This shows that the S-ECC saliva is less specialized in proinflammatory cytokines and conversely expresses inflammatory cytokines which results in chronic inflammation. The occurrence of chronic inflammation is caused because innate immunity in S-ECC is not as good as in free caries so innate immunity is not able to fight the pathogens that cause dental caries.

IL-10 is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen-associated molecular patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, and abscesses.

The results showed the occurrence of decreased IL-10 expression in preschool children with S-ECC compared with in caries-free children. This may be preschool children with S-ECC responding to more antigens in the form of *S. mutans* bacteria, which are relatively high in number compared to children with free caries.[11]

Antigen structures called PAMPs, which will be recognized by PRRs, namely TLRs, are very important to trigger the effect or phase of the innate immune response.[12] TLR2 and TLR4 involved in the introduction of gram-positive and gram-negative bacteria that have been detected in the odontoblast cell membrane in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through dentinal tubules during carious infection.[13]

One of the main consequences of TLR activation is an increase in innate immune efficacy, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] One of the main consequences of TLR activation is an increase in the effectiveness of innate immunity, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] This causes a prolonged increase in inflammatory cytokines in S-ECC, increasing IFN-γ increase expression.[15] which can ultimately cause oral cavity tissue damage that affects general health, ranging from local pain, infections, abscesses, difficulty chewing, malnutrition, indigestion, and trouble sleeping.[16]

Study shows that an increase in pro-inflammatory cytokines occurs in S-ECC; this must be balanced by the host immune system by producing anti-inflammatory cytokines, IL-10. Cluster differentiation 4 ($CD4^+$) memory T cells are developed in response to pathogenic microbes. $CD4^+$ memory T cells prevent the body from fighting pathogens.[17] $CD4^+$ cells also respond as antipathogens,[18] which produce antibodies and cytotoxicity of cluster differentiation 8 ($CD8^+$) T cells,[19] but this does not occur in S-ECC so IL-10 expression in S-ECC saliva is lower than in caries-free children. This study requires larger sample size to evaluate the expression in different age groups and populations.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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Original Research

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Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the SPSS Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

The data obtained indicate that the average IL-10 in the caries-free group was higher than the S-ECC group, but the difference was not statistically significant between caries-free and S-ECC.

Discussion

IL-10 is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen-associated molecular patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, and abscesses.

The results showed the occurrence of decreased IL-10 expression in preschool children with S-ECC compared

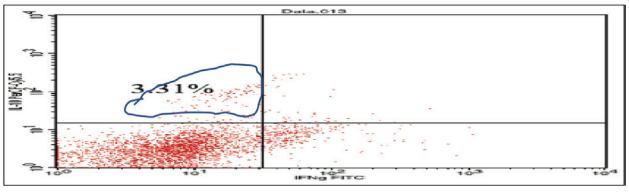

Figure 1: Expression of interleukin-10 (3.31%) from severe early childhood caries salivary after analyzed by flow-cytometry test

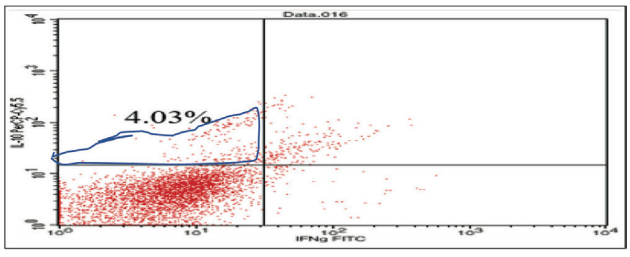

Figure 2: Expression of interleukin-10 (4.03%) from caries free salivary after analyzed by flow-cytometry test

AQ2

Table 1: Normality test using Shapiro–Wilk interleukin-10 expression from severe early childhood caries and caries free

Variable	Kolmogorov–Smirnov			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
	143	16	200	970	16	844

IL-10 = interleukin-10, Df = degrees of freedom

AQ2

Table 2: Mean and standard deviation of interleukin-10 expression in severe early childhood caries and caries free analyzed by flow-cytometry test, which was tested using independent *t* test

Group	*n*	IL-10 expression (%)	
		Mean ± SD	*P*
Caries free	8	4.04 ± 0.89	0.11
S-ECC	8	3.32 ± 0.76	

S-ECC = severe early childhood caries, SD = standard deviation, IL-10 = interleukin-10

with in caries-free children. This may be preschool children with S-ECC responding to more antigens in the form of *S. mutans* bacteria, which are relatively high in number compared to children with free caries.[11]

Antigen structures called PAMPs, which will be recognized by PRRs, namely TLRs, are very important to trigger the effect or phase of the innate immune response.[12] TLR2 and TLR4 involved in the introduction of gram-positive and gram-negative bacteria that have been detected in the odontoblast cell membrane in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through dentinal tubules during carious infection.[13]

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Study shows that an increase in pro-inflammatory cytokines occurs in S-ECC; this must be balanced by the host immune system by producing anti-inflammatory cytokines, IL-10. Cluster differentiation 4 ($CD4^+$) memory T cells are developed in response to pathogenic microbes. $CD4^+$ memory T cells prevent the body from fighting pathogens.[17] $CD4^+$ cells also respond as antipathogens,[18] which produce antibodies and cytotoxicity of cluster differentiation 8 ($CD8^+$) T cells,[19] but this does not occur in S-ECC so IL-10 expression in S-ECC saliva is lower than in caries-free children. This study requires larger sample size to evaluate the expression in different age groups and populations.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions.

Financial support and sponsorship

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AQ2: Please cite or indicate Tables 1 and 2 in the text where they have been described.

Original Research

Analysis of Interleukin-10 Anti-inflammatory Cytokines in Salivary Lymphocyte Surface: A Pilot Study

AQ1, 2

Retno Indrawati[1], Muhammad Luthfi[1], Aqsa S. Oki[1], Yuliati[1,2], Agung Sosiawan[3], Priyawan Rachmadi[4], Muhaimin Rifai[5]

AQ3

[1]Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, [3]Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, [4]Department of Physiology, Cell Culture and Animal Development, Faculty of Science, Universitas Brawijaya, Malang, Indonesia, [5]xxxx

Abstract

Aim: The aim of this study was to analyze the expression of interleukin-10 (IL-10) in children with severe early childhood caries (S-ECC) and caries-free children. **Materials and Methods:** This was an observational analytic pilot study performed on children with social factors-ECC (S-ECC), and caries-free children as the objects of research with a cross-sectional study design. Saliva of children aged 4–6 years from the group of caries children in severe and caries-free early childhood was taken. Samples were taken by rinsing with 1.5% sterile NaCl for 30 s and then accommodated in a sterile tube, to get a 40 mL sample from the aforementioned procedure repeated four times. Flow-cytometry test was used to analyze the IL-10 expression. The results of the study were analyzed using the normality test using Shapiro–Wilk, then continued with *t* test using the Statistical Package for the Social Sciences (SPSS) software program, version 20.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by independent *t* test to see the difference between caries-free children and S-ECC. **Results:** The expression of IL-10 in the saliva of children with severe ECC was 3.32 ± 0.79; meanwhile, in caries-free children it was 4.04 ± 0.65. **Conclusion:** The IL-10 expression in children with severe ECC was significantly lower than that of in caries-free children.

Keywords: Interleukin-10 Anti-inflammatory Cytokines, Lymphocyte Cells, Severe Early Childhood Caries

Received: 20-11-2018, **Revised:** 13-11-2019, **Accepted:** 04-03-2020, **Published:** XX-XX-XXXX

Introduction

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The immune system is a very varied compilation of cells, consisting of two parts of the immune system, namely innate and adaptive. The innate and adaptive immune systems are interrelated, and recognition by innate immune systems can cause the activation of the adaptive immune response.[3] The innate immune system is the first line of host defense against pathogens and recognizes molecules repeatedly against pathogens, which are called pathogen-related molecular patterns through germline-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[4]

The components that regulate the immune system, such as an immune regulator cells and regulating cytokines, both natural and acquired as induced by an antigen, plays an important role in controlling various immune responses, both physiological and pathological. Local and systemic interleukin-10 (IL-10) responses have been shown to have pathophysiological relevance in several diseases such as malignancy, infectious diseases, autoimmune diseases, and atopic disorders.[5] Because of

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How to cite this article: Indrawati R, Luthfi M, Oki AS, Yuliati, Sosiawan A, Rachmadi P, *et al*. Analysis of interleukin-10 anti-inflammatory cytokines in salivary lymphocyte surface: A pilot study. J Int Oral Health 2020;XX:XX-XX.

this, IL-10 can activate signal transducer and activator of transcription 3 (STAT3) in macrophages and T cells to restore and respond to the presence of pro-inflammatory cytokines.[6]

Immunity in the oral cavity's immune system has an important role that is balancing the amount of microbes in the mouth. The microbial activity in oral cavity can be fluctuatives, due to pathogen situations. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system. The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine, which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines that function to limit tissue damage and to maintain tissue homeostasis.[7] IL-10 is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

For dental caries preventions, many efforts had been carries out. The government and supporting health organization ran some prevention programs, such as dental counseling to community. Most of the programs targeted children, pregnant woman, and elderly. They teach how to brush teeth properly, dietary that good for dental health, prevention treatment that can be applied for children, and vaccines.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of social factors-ECC (S-ECC).

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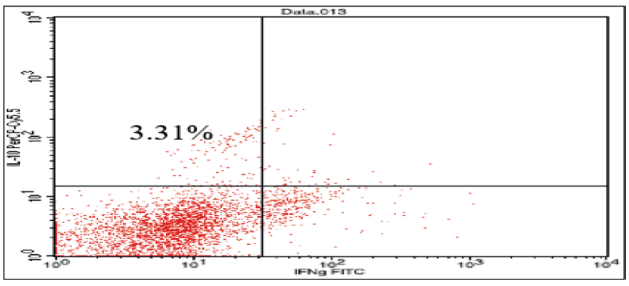

Figure 1: Expression of interleukin-10 from severe early childhood caries salivary after analyzed by flow-cytometry test

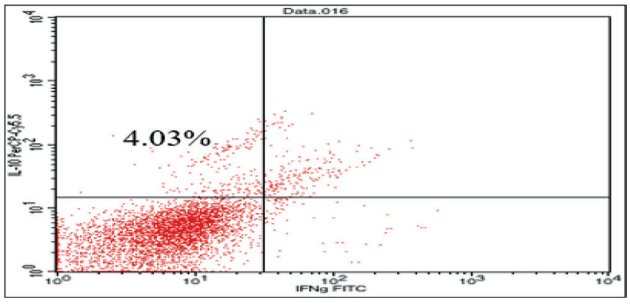

Figure 2: Expression of interleukin-10 from caries free salivary after analyzed by flow-cytometry test

AQ4, 5

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Variable	Kolmogorov–Smirnov[a]			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
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AQ5

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Original Research

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Muhammad Luthfi[1], Retno Indrawati[1], Aqsa S. Oki[1], Priyawan Rachmadi[2], Muhaimin Rifai[3]

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This was an observational analytic study using children with S-ECC and caries-free children as the objects of research with a cross-sectional study design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 209/HRECC.FODM/IX/2017).

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Group one were children with a diagnosis of S-ECC characterized by decay, extraction, and filling (def-t > 6), whereas, the second group were preschool children who were diagnosed with free caries marked with def-t = 0.

5 mL saliva is taken from preschool children with S-ECC and caries free. Sampling was carried out by researchers and trained research assistants using standard protocols. Subjects were asked not to consume food and drink, or brush their teeth for 60 min before the study was conducted. The samples obtained were stored at −80°C for analysis. IL-10 expression was analyzed using flow cytometry, according to Luthfi *et al.*[10]

Statistical analysis: The data were analyzed by independent t test to see the difference between caries free and S-ECC.

Results

Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

The data obtained indicate that the average IL-10 in the caries-free group was higher than the S-ECC group, but the difference was not statistically significant between caries-free and S-ECC.

Discussion

IL-10 is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen-associated molecular patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, and abscesses.

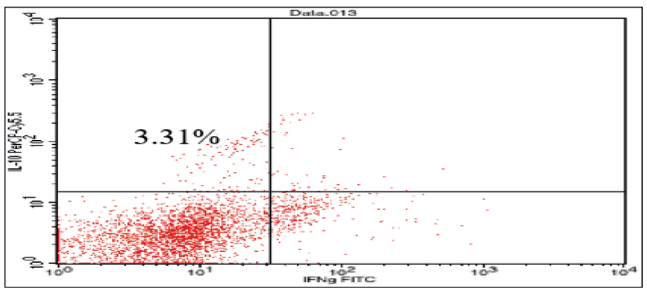

Figure 1: Expression of interleukin-10 from severe early childhood caries salivary after analyzed by flow-cytometry test

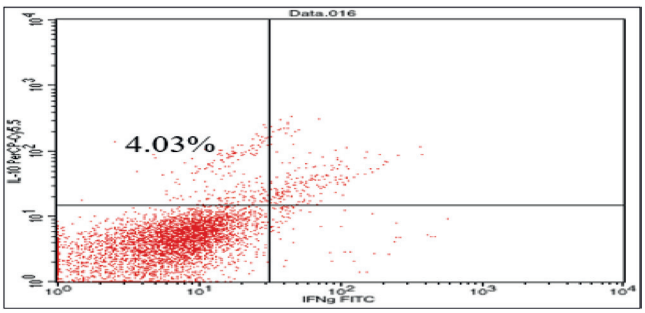

Figure 2: Expression of interleukin-10 from caries free salivary after analyzed by flow-cytometry test

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Table 1: Normality test using Shapiro–Wilk interleukin-10 expression from severe early childhood caries and caries free

Variable	Kolmogorov–Smirnov[a]			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
	143	16	200*	970	16	844

IL-10 = interleukin-10, Df = degrees of freedom

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Table 2: Mean and standard deviation of interleukin-10 expression in severe early childhood caries and caries free analyzed by flow-cytometry test, which was tested using independent *t* test

Group	*n*	IL-10 expression (%)	
		Mean ± SD	*P*
Caries free	8	4.04 ± 0.89	0.11
S-ECC	8	3.32 ± 0.76	

S-ECC = severe early childhood caries, SD = standard deviation, IL-10 = interleukin-10

The results showed the occurrence of decreased IL-10 expression in preschool children with S-ECC compared with in caries-free children. This may be preschool children with S-ECC responding to more antigens in the form of *S. mutans* bacteria, which are relatively high in number compared to children with free caries.[11]

Antigen structures called PAMPs, which will be recognized by PRRs, namely TLRs, are very important to trigger the effect or phase of the innate immune response.[12] TLR2 and TLR4 involved in the introduction of gram-positive and gram-negative bacteria that have been detected in the odontoblast cell membrane in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through dentinal tubules during carious infection.[13]

One of the main consequences of TLR activation is an increase in innate immune efficacy, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] One of the main consequences of TLR activation is an increase in the effectiveness of innate immunity, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] This causes a prolonged increase in inflammatory cytokines in S-ECC, increasing IFN-γ increase expression.[15] which can ultimately cause tissue damage that affects general health, ranging from local pain, infections, abscesses, difficulty chewing, malnutrition, indigestion, and trouble sleeping.[16]

AQ9

Study shows that an increase in pro-inflammatory cytokines occurs in S-ECC; this must be balanced by the host immune system by producing anti-inflammatory cytokines, IL-10. Cluster differentiation 4 ($CD4^+$) memory T cells are developed in response to pathogenic microbes. $CD4^+$ memory T cells prevent the body from fighting pathogens.[17] $CD4^+$ cells also respond as antipathogens,[8] which produce antibodies and cytotoxicity of cluster differentiation 8 ($CD8^+$) T cells,[19] but this does not occur in S-ECC so IL-10 expression in S-ECC saliva is lower than in caries-free children. This study requires larger sample size to evaluate the expression in different age groups and populations.

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Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions.

Financial support and sponsorship

This study was supported by 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.

Conflicts of interest

There are no conflicts of interest.

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Original Research

Analysis of Interleukin-10 Anti-inflammatory Cytokines in Salivary Lymphocyte Surface: A Pilot Study

Muhammad Luthfi[1], Retno Indrawati[1], Aqsa S. Oki[1], Priyawan Rachmadi[2], Muhaimin Rifai[3]

[1]Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, [2]Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, [3]Department of Physiology, Cell Culture and Animal Development, Universitas Brawijaya, Kota Malang, Jawa Timur, Indonesia

Abstract

Aim: The aim of this study was to analyze the expression of interleukin-10 (IL-10) in children with severe early childhood caries (ECC) and caries-free children. **Materials and Methods:** This was an observational analytic pilot study performed on children with social factors-ECC (S-ECC), and caries-free children as the objects of research with a cross-sectional study design. Saliva of children aged 4–6 years from the group of caries children in severe and caries-free early childhood was taken. Samples were taken by rinsing with 1.5% sterile NaCl for 30 s and then accommodated in a sterile tube, to get a 40 mL sample from the aforementioned procedure repeated four times. Flow-cytometry test was used to analyze the IL-10 expression. The results of the study were analyzed using the normality test using Shapiro–Wilk, then continued with *t* test using the Statistical Package for the Social Sciences (SPSS) software program, version 20.0 (IBM, New York). The data were analyzed by independent *t* test to see the difference between caries-free children and S-ECC. **Results:** The expression of IL-10 in the saliva of children with severe ECC was 3.32 ± 0.79; meanwhile, in caries-free children it was 4.04 ± 0.65. **Conclusion:** The IL-10 expression in children with severe ECC was significantly lower than that of in caries-free children.

Keywords: Interleukin-10, Lymphocyte Cells, Severe Early Childhood Caries

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Introduction

Dental caries is a multifactorial disease due to various factors, namely cariogenic microbes, carbohydrates, and social factors, whereas early childhood caries (ECC) is often found in children with low-socioeconomic conditions.[1] Several studies have recognized the importance of infection of *Streptococci mutans*.[2]

The immune system is a very varied compilation of cells, consisting of two parts of the immune system, namely innate and adaptive. The innate and adaptive immune systems are interrelated, and recognition by innate immune systems can cause the activation of the adaptive immune response.[3] The innate immune system is the first line of host defense against pathogens and recognizes molecules repeatedly against pathogens, which are called pathogen-related molecular patterns through germline-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[4]

The components that regulate the immune system, such as an immune regulator cells and regulating cytokines, both natural and acquired as induced by an antigen, plays an important role in controlling various immune responses, both physiological and pathological. Local and systemic interleukin-10 (IL-10) responses have been shown to have pathophysiological relevance in several diseases such as malignancy, infectious diseases, autoimmune diseases, and atopic disorders.[5] Because of this, IL-10 can activate signal transducer and activator of transcription 3 (STAT3) in macrophages and T cells to restore and respond to the presence of pro-inflammatory cytokines.[6]

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Immunity in the oral cavity's immune system has an important role that is balancing the amount of microbes in the mouth. The microbial activity in oral cavity can be fluctuatives, due to pathogen situations. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system. The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine, which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines that function to limit tissue damage and to maintain tissue homeostasis.[7] IL-10 is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

For dental caries preventions, many efforts had been carries out. The government and supporting health organization ran some prevention programs, such as dental counseling to community. Most of the programs targeted children, pregnant woman, and elderly. They teach how to brush teeth properly, dietary that good for dental health, prevention treatment that can be applied for children, and vaccines.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of social factors-ECC (S-ECC).

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Statistical analysis: The data were analyzed by independent t test to see the difference between caries free and S-ECC.

Results

Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

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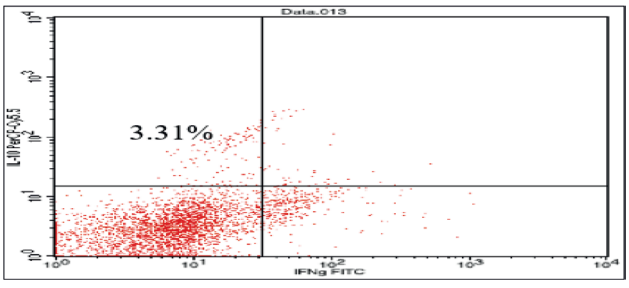

Figure 1: Expression of interleukin-10 from severe early childhood caries salivary after analyzed by flow-cytometry test

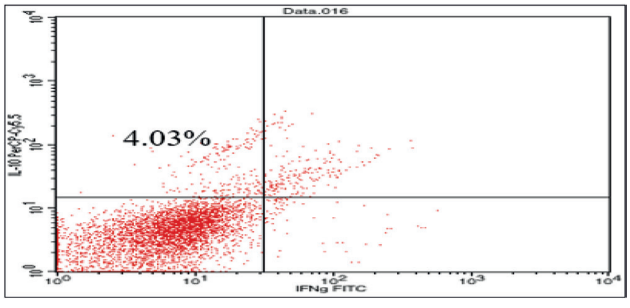

Figure 2: Expression of interleukin-10 from caries free salivary after analyzed by flow-cytometry test

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Variable	Kolmogorov–Smirnov[a]			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
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IL-10 = interleukin-10, Df = degrees of freedom

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		Mean ± SD	*P*
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Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions.

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Analysis of IL-10 Anti-Inflammatory Cytokines Expression in Saliva of Children with Severe Early Childhood Caries and Caries-Free Children

By Udijanto Tedjosasongko

ANALYSIS OF IL-10 ANTI-INFLAMMATORY CYTOKINES EXPRESSION IN SALIVA OF SEVERE EARLY CHILDHOOD CARIES

Running title: IL-10 CYTOKINES EXPRESSION IN SALIVA OF CARIES

Abstract:

Objective: This study aimed to analyze the expression of IL-10 in children with severe early childhood caries and caries-free children.

Materials and Method: Saliva taken from preschool aged children (4 to 6 years) was divided into two groups, ie heavy caries group with dmft> 6 and caries free with dmft = 0. Salivary lymphocyte cells were obtained from participants by rinsing their oral cavities with 10 mL of sterile and 1.5% NaCl solution while they gargled without swallowing for 30 s before expectorating the resulting uid into a sterile glass – a procedure repeated four times. The collected solution was centrifuged (15 min at 450 g) at 4°C and the pellets then mixed with 2 mL of Roswell Park Memorial Institute medium. For expression test of IL-10, flow cytometry test was used.

Statistical test used: The results were listed as the mean ± standard deviation. All statistical analyses were performed using SPSS 20 (IBM, New York, USA). The statistical difference was analyzed by *one way anova*. Correlation analyses were performed using tukey HSD with $P < 0.05$ being considered to be significant.

Results: IL-10 expression in saliva of children with severe early childhood caries was 3.32±0.79. Whereas, in children with no caries was 4.04±0.65.

Conclusion: IL-10 expression in children with severe early childhood caries was significantly lower than in caries-free children.

Key-Words: Severe Early Childhood Caries, Il-10, Lymphocyte Cells

Key Messages

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen associated moleculer patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, abscesses

Introduction:

Caries in early childhood (ECC) is a multifactorial disease resulting from interactions of various factors, namely: cariogenic microbes, exposure to carbohydrate fermentation and various social variables. ECC is a condition of health abnormalities found in children living in socially disadvantaged communities, such as malnourished people with social and health inequalities.[1] ECC has an impact on general health, ranging from local pain, infections, abscesses, difficulty in chewing, malnutrition, indigestion, and insomnia.[2]

The body's immune system functions to defend the human body from foreign inventors. A compromised immune system can cause various diseases, such as infection, aging, allergies, various organ disorders and other diseases, such as cancer and auto immune deficiency syndrome (AIDS).[3] The role of the body's immune system is becoming increasingly important in understanding the mechanisms of disease prevention. The effective function of the body's immune system is to immediately eradicate the infectious agent from the body. This is done by an interactive system of actions, namely innate (very specific), fast but non-specific and adaptive immune system.[4] which function as a pathogenic killer[5], produce antibodies, and CD8 + T cell cytotoxicity[6].

Immunity in the oral cavity is a system that makes a balance by controlling various microbes in the oral cavity that are fluctuating due to external aggression. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system.

The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines which function to limit tissue damage and to maintain tissue homeostasis.[7] Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

Various preventions of dental caries had been carried out, for example by brushing teeth properly, fluoridating with topical applications, and making vaccines which until now have not shown the expected results.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of early detection of S-ECC.

Materials and Methods:

Study Design:

This was an observational analytic study using children with severe early childhood caries and caries-free children as the objects of research with a cross sectional study design. Ethical clearance test at Universitas Airlangga, Faculty of Dental Medicine was done with Health Research Ethical Clearance Commission number of 209/HRECC. FODM/IX/2017.

Sampling Criteria:

Eight children with S-ECC and eight free of caries were taken from the saliva of kindergarten children aged 4 to 6 years in the south Surabaya region which were previously divided into two groups.

Group one was children with who were diagnosed with severe early childhood caries (S-ECC) marked by decay exfoliation and filling (def-t>6). Whereas, group two was kindergarten children diagnosed with free caries marked with def-t = 0.

5ml saliva taken from the kindergarten children with SECC and caries-free . Sampling was carried out by researchers and trained personnel using protocol standards. Subjects might not eat, drink, chew gum, or brush their teeth for 60 minutes before sampling. Furthermore, samples were frozen at -80oC for analysis.

Observational parameters:

Analysis of IL-10 expression was determined using flow cytometry, according to Luthfi *et al* (2019)[10a]. 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 optimal concentration of mAbs is determined for each mAb with titration. Flow cytometry simultaneously measures and analyzes the physical properties of particles such as cells because it flows through the flow of fluid through a beam of light. The nature of scattering cell light can be used to analyze changes in size, granularity, internal complexity and relative fluorescence intensity. Flow cytometry analysis was performed to determine directly the pattern of lymphocyte immunomodulation, using conjugated monoclonal antibodies (mAbs).

Statistical Analysis:

The results were listed as the mean ± standard deviation. All statistical analyses were performed using SPSS 20 (IBM, New York, USA). The statistical difference was analyzed by t Test.

Results:

Data from the results of the study before the test using the t test, conducted tests of normality and homogeneity using the Shapiro-Wilk test. From the results of these tests indicate a value of P> 0.05 which means that all data are normally distributed and homogeneous. Normality test using Shapiro Wilk shows) data with normal distribution with p = 0.844 (p> 0.05, while Levene test results show homogeneous data with p = 0.726 (p> 0.05).

The data obtained showed that the average IL-10 in the caries free group was higher than the S-ECC group, but the difference was not statistically significant between caries free and S-ECC

Discussion:

The results of the study showed that there was expression of IL-10 decrease in children with S-ECC compared to that in caries-free children. This was probably due to the S-ECC patients responding more antigens in the form of S. mutans bacteria which were relatively high in numbers compared to that in children with free caries.[10b]

An antigen structure called Pathogen Associated Molecular Pattern (PAMPs), which will be recognized by Pattern Recognition Receptors (PRRs), namely Toll-like receptors (TLRs), is very important for triggering the effector phase of the innate immune response.[11] TLR2 and TLR4 involved in the introduction of Gram-positive and Gram-negative bacteria that have been detected in odontoblast cell membranes in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through the dentinal tubules during carious infection.[12] One of the main consequences of TLR activation is an increase in the efficacy of innate immunity, including antimicrobial agents and proinflammatory cytokines and chemokines that recruite and activate immune cells.[13] One of the main consequences of TLR activation is increased efficacy of innate immunity, including antimicrobial agents and proinflammatory cytokines and chemokines that recruit and activate immune cells.[13] This causes the S-ECC saliva to increase prolonged inflammatory cytokines, IFN-γ expression increase.[14] which eventually can cause tissue damage that affects health in general, starting from local pain, infection, abscess, difficulty in chewing, malnutrition, indigestion, and sleeping difficulty.[15].

Based on the results of this study, high expression of proinflammatory cytokines in S-ECC should be balanced by the immune host system by producing anti-inflammatory cytokines, IL-10. As a response to pathogenic microbes, the body's adaptive immune system develops effector cells that function to prevent these threats, namely CD4 + memory T cells which

serve as a protective against bacterial infections.[16] CD4 + cells participate in responding to secondary infections that have the potential as anti-pathogens [5] producing antibodies and CD8 + T-cell cytotoxicity.[6] However, this did not occur in S-ECC so IL-10 expression in S-ECC saliva was lower than that in caries-free children. This was probably due to the role of the immune system in S-ECC which was not as good as that in caries-free children.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than caries-free

Acknowledgement: Department of Oral Biology, Faculty of Dentistry, Universitas Airlangga

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

Conflict of interest: There is no conflict of interest in this research

Author 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 Aqsa Sjuhada Oki: Study concept, clinical studies, experimental studies, data analysis, manuscript review Retno Indrawati: data interpretation, Statistical analysis, manuscript preparation, manuscript editing, Priyawan Rachmadi: Statistical analysis, manuscript preparation, manuscript editing, Muhaimin Rifa'i: manuscript editing, manuscript review

Patient declaration of consent: Before saliva sampling from children aged 4 to 6 years, parents of the sample had agreed to signed a written informed consent.

Data Availability statement: Dataset can be made available after embargo period due to commercial restrictions

Abbreviations

ECC	**:** Early childhood caries
S-ECC	: Severe early childhood caries
IL-10	: Interleukin-10
PRRs	: Pathogen recognition receptors

TLR : Toll like receptors
PAMPs : Pathogen associated moleculer patterns
CD8 : Cluster differentiation 8
CD4 : Cluster differentiation 4
def-t : Decay exfoliation and filling
FITC : Fluorescein isothiocyanate
PE : Phycoerythrin
APC : Allophycocyanin
PerCP : Peridinin chlorophyll protein

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Table 1. Normality test uses Shapiro Wilk IL-10 expression from S-ECC and caries free

variable	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
IL10	Statistic	Df	Sig.	Statistic	Df	Sig.
	,143	16	,200*	,970	16	,844

Table 2. Mean and standard deviation of IL-10 expression in S-ECC and caries free analyzed by flow cytometry test which was tested using independent t Test

Group	n	IL-10 Expression (%)	
		Mean ± SD	P
Caries Free	8	4,04 ± 0,89	0,11
S-ECC	8	3,32 ± 0,76	

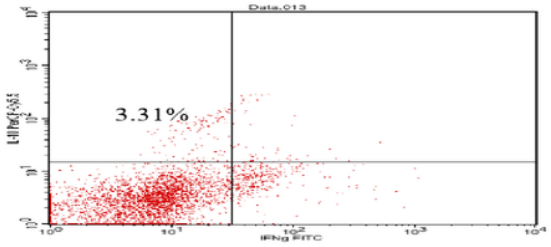

Figure 1. Expression of IL-10 from S-ECC salivary after analyzed by Flow Cytometry test.

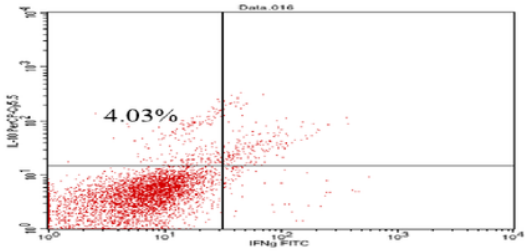

Figure 2. Expression of IL-10 from caries free salivary (B) after analyzed by Flow Cytometry test.

Analysis of IL-10 Anti-Inflammatory Cytokines Expression in Saliva of Children with Severe Early Childhood Caries and Caries-Free Children

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ANALYSIS OF IL-10 ANTI-INFLAMMATORY CYTOKINES EXPRESSION IN SALIVA OF SEVERE EARLY CHILDHOOD CARIES

Muhammad Luthfi[1], Aqsa Sjuhada Oki [2], Retno Indrawati[3], Priyawan Rachmadi[4], Muhaimin Rifa'i[5]

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[4]Departments of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia
[5]Department of Imunology *and Physiology, Faculty of Sciences, Brawijaya University, Malang- Indonesia*

Correspondence: Muhammad Luthfi, Departement of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia,.

Running title: IL-10 CYTOKINES EXPRESSION IN SALIVA OF CARIES

Abstract:

Objective: This study aimed to analyze the expression of IL-10 in children with severe early childhood caries and caries-free children.

Method: Saliva taken from preschool aged children (4 to 6 years) was divided into two groups, ie heavy caries group with dmft> 6 and caries free with dmft = 0. Salivary neutrophils were obtained from participants by rinsing their oral cavities with 10 mL of sterile and 1.5% NaCl solution while they gargled without swallowing for 30 s before expectorating the resulting uid into a sterile glass – a procedure repeated four times. The collected solution was centrifuged (15 min at 450 g) at 4°C and the pellets then mixed with 2 mL of Roswell Park Memorial Institute medium. For expression test of IL-10, flow cytometry test was used.

Statistical test used: The results were listed as the mean ± standard deviation. All statistical analyses were performed using SPSS 20 (IBM, New York, USA). The statistical difference was analyzed by *one way anova*. Correlation analyses were performed using tukey HSD with $P < 0.05$ being considered to be significant.

Results: IL-10 expression in saliva of children with severe early childhood caries was 3.32±0.79. Whereas, in children with no caries was 4.04±0.65.

Conclusion: IL-10 expression in children with severe early childhood caries was significantly lower than in caries-free children.

Key-Words: Severe Early Childhood Caries, Il-10, Lymphocyte Cells

Key Messages

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen associated moleculer

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patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, abscesses

Introduction:

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Results:

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Data from the results of the study before the test using the t test, conducted tests of normality and homogeneity using the Shapiro-Wilk test. From the results of these tests indicate a value of P> 0.05 which means that all data are normally distributed and homogeneous.

The results of statistical analysis one way anova in the S-ECC group showed that IL-10 expression (3.31%) was lower than that of the caries-free group (4.03%) (Table 1). Based on Levene's test, there was significant difference in IL-10 expression between S-ECC with caries-free.

Discussion:

The results of the study showed that there was expression of IL-10 decrease in children with S-ECC compared to that in caries-free children. This was probably due to the S-ECC patients responding more antigens in the form of S. mutans bacteria which were relatively high in numbers compared to that in children with free caries.[10]

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Acknowledgement: Department of Oral Biology, Faculty of Dentistry, Universitas Airlangga

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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.

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  - Add limitation and future scope at end of discussion. If added highlight one.

Conflict of interest:

There is no conflict of interest in this research

Author 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

Aqsa Sjuhada Oki: Study concept, clinical studies, experimental studies, data analysis, manuscript review

Retno Indrawati: data interpretation, Statistical analysis, manuscript preparation, manuscript editing,

Priyawan Rachmadi: Statistical analysis, manuscript preparation, manuscript editing,

Muhaimin Rifa'i: manuscript editing, manuscript review

Patient declaration of consent:

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Dataset can be made available after embargo period due to commercial restrictions

Abbreviations

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PRRs	: Pathogen recognition receptors
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PAMPs	: Pathogen associated moleculer patterns
CD8	: Cluster differentiation 8
CD4	: Cluster differentiation 4
def-t	: Decay exfoliation and filling

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Table 1. Mean and standard deviation of IL-10 expression in caries free and S-ECC analyzed by flow cytometry test and t test statistic test

Groups	N	IL-10 Expression (%)		
		Mean	Standard Deviation	Standard error mean
S-ECC	8	3.321	0.787	0.278
Caries-free	8	4.044	0.648	0.229

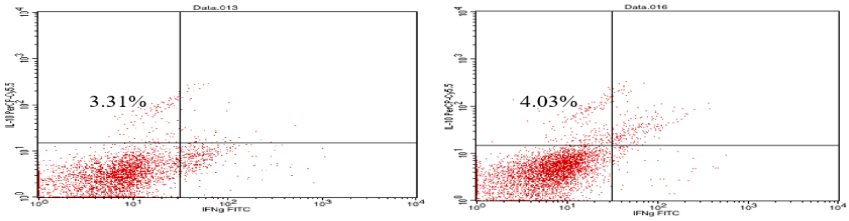

Figure 1. Expression of IL-10 from S-ECC saliva after analyzed by Flow Cytometry test.

Original Research

Effectiveness of Okra Fruit (*Abelmoschus esculentus*) Extract Against *Aggregatibacter actinomycetemcomitans* (*Aa*) as a Bacterium that Causes Aggressive Periodontitis

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Abstract

Aims and Objectives: The aim of this study was to determine that okra fruit extracts are effective in inhibiting growth and killing the *Aggregatibacter actinomycetemcomitans* (*Aa*) bacteria that cause aggressive periodontitis. **Materials and Methods:** *Aa* ATCC 4371 strain Y3 serotype b bacteria obtained from the Stock Research Center of the Faculty of Medicine, Airlangga University, Jawa Timur, Indonesia, were bred on the Mueller Hinton media with the inclusion criteria that identification of bacteria from the stock shows that the bacterium is *Aa*, and the growth of bacteria in the Mueller Hinton media is with a number of colonies between 30–300 colony forming units (CFU)/mL. Culture media containing *Aa* bacteria were incubated for 1 × 24 h at 37°C, after it was diluted according to McFarland standard 0.5 (1.5 × 108 CFU/mL). Fresh okra fruit derived from Materia Medica was prepared for extract. Serial dilution or dilution methods of 1:2 (wt/vol) are used for the detection of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **Results:** One-way analysis of variance test showed a difference with significance (P = 0.000), whereas, Tukey honestly significant difference (HSD) test showed a significant difference between okra fruit extract group with positive control concentrations of 100%, 3.125%, and 1.565%. **Conclusion:** The okra fruit extract effectively kills the *Aa* bacteria that causes aggressive periodontitis, as indicated by MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

Keywords: *Aggregatibacter Actinomycetemcomitans*, Aggressive Periodontitis, Minimal Bactericidal Concentration, Minimal Inhibitory Concentration, okra fruit (*Abelmoschus esculentus*) extract

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Introduction

Periodontitis is an inflammation that affects the supporting tissues of teeth, which is caused by microorganisms, and can cause progressive damage to the periodontal ligament, alveolar bone, and is accompanied by pocket formation. Periodontitis causes permanent tissue destruction, characterized by chronic inflammation, migration of the fused epithelium to the apical, loss of connective tissue, and loss of alveolar bone.[1]

Aggressive periodontitis (AP) is a complex disease, which is caused by microbial changes and cellular dysfunction, and is characterized by a rapid loss of attachment and bone damage to the tooth surface.[2] The majority of periodontal pathogens are Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (*Aa*), which has often been associated with AP.[3] The role of this bacterium in the pathogenesis of periodontitis is due to its ability to attach to epithelial cells and produce many virulent factors such as extracellular matrix proteins, proteases, collagenase, endotoxin (LPS), bacteriocins, hemotactic inhibitors, leukotoxins, cytotoxins, toxic metabolic substances, and immunosuppressive proteins.[4]

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The use of synthetic drugs is not only expensive for the treatment of a disease, but also has toxicity and adverse side effects. This type of situation causes the need to look for new drug alternatives to treat a disease. Herbal alternatives have enormous potential to develop new drugs that are very useful for treatment and are strong and effective antibacterial agents.[5]

Abelmoschus esculentus (okra) has many benefits. This is because okra contains secondary metabolite components, such as alkaloids, terpenoids, and flavonoids.[6] Flavonoids found in plants are known for their antibacterial effects because of their ability to reduce the permeability of bacterial cell walls.[7]

Because of the explanation of aforementioned fact, the researchers decided to prove that okra fruit extract was effective in inhibiting and killing *Aa* bacteria that cause AP. From the results of this research, it is expected to be used as a therapy for AP.

Materials and Methods

Setting and design

This was an experimental laboratory using a posttest only control group design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 112/HRECC. FODM/VII/2018).

Sampling criteria

This study uses *Aa*, ATCC 4371 strain Y3 serotype b bacteria, obtained from the Stock Research Center of the Faculty of Medicine, Airlangga University, Jawa Timur, Indonesia, with specification of ATCC 43718, which were bred on the Mueller Hinton media with the inclusion criteria that the identification of bacteria from the stock shows that the bacterium is *Aa* and the bacterial growth in the Mueller Hinton media is with a number of colonies between 30 and 300 colony forming units (CFU)/mL.

Study method

Aa ATCC 4371 strain Y3 serotype bacterial stock was inoculated in the brain heart infusion broth (BHIB) culture media. Culture media containing *Aa* bacteria was incubated for 1 × 24 h at 37°C, after which it was diluted according to McFarland standard 0.5 (1.5 × 108 CFU/mL). Furthermore, the bacteria were ready to be tested.

Okra fruit extract making

Fresh okra fruit derived from Materia Medica for extract was prepared.[7] Samples of okra fruit were cut into pieces and weighed 200 g, then put into a jar, and 70% of ethanol was added to make the volume to 300 mL. Maceration was carried out for 24 h at room temperature. After 24 h, the solution was filtered or separated using a Buchner filter. Filtering residue was aerated, and maceration was done up to three times. The sieve 1–3 was mixed and concentrated with a rotary vacuum evaporator at 40°C until a concentrated extract was obtained. To obtain various concentrations, serial dilution or dilution methods of 1:2 (wt/vol) were used.

Antibacterial test using the serial dilution method

Preparation of *Aa* bacteria stored in BHIB media in an incubator at 37°C was obtained with a sterile Ose needle.[8] The Mueller Hinton media was embedded by scratching. The bacteria that had been scratched on Mueller Hinton media were incubated in an incubator at 37°C for 1 × 24 h. The scratched bacteria were obtained from the Mueller Hinton media using a sterile Ose needle. It was put in the BHIB until the turbidity was the same as the McFarland 0.5 standard. Eleven sterile test tubes were prepared. Each test tube was labeled 1–9 (concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, and 0.39%, respectively), then tube 10 was given K(+) label, which was a positive control. Tube 10 contained the bacterial suspension, which was equivalent to McFarland 0.5 turbidity standard. Tube 11 was labeled with K(−), which was a negative control. This tube contained okra fruit extract with a concentration of 100%. The tube 1 was filled with 4 mL concentration of 100% okra fruit extract. The tubes 2–9 were filled with 2 mL of BHIB liquid media. Two milliliter of solution from the tube 1 was put in tube 2. It was mixed until homogeneous, so that the concentration of 50% was obtained. The same thing was carried out up to tube 9 until all extract concentrations were obtained with a ratio of 1:2 (wt/vol). To test turbidity, bacterial suspension media were taken, which had been equalized with McFarland 0.5 turbidity standard of 0.1 mL and put into test tubes in 1–9 labels (concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, and 0.39%, respectively). Then, all the tubes that were put in an airtight anaerobic container were then incubated at 37°C for 1 × 24 h. After one incubation, turbidity was observed. If the turbidity of the tube was still equivalent or more turbid than the positive control (K+) tube containing the bacterial suspension McFarland 0.5, it meant that bacteria can still thrive. However, when the solution in the tube appeared to be clearer than the K (+) tube, it meant that the growth of bacteria began to be inhibited. This was what showed the minimum inhibition concentration (MIC). After observing turbidity, a total plate count (TPC) test was conducted to determine bacteriostatic and bacteriocide properties. The TPC test was carried out on Mueller Hinton agar media containing concentrations of extracts from tubes that looked the clearest. Furthermore, each petri dish was incubated at 37°C for 1 × 24 h. The number of colonies was then counted.

Statistical analysis

The data obtained were the number of bacterial colonies measured in CFU. Data were then tabulated and analyzed

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using the Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM, New York).

The data distribution was carried out with the Kolmogorov–Smirnov test to determine whether the data could be normally distributed. To identify whether the collected data were homogeneous, a variance homogeneity test was performed using the Levene test with $\alpha > 0.05$. Furthermore, the parametric test using the analysis of variance (ANOVA) was used to identify the significance of differences in the number of bacterial colonies between the study groups. All analyses were tested at the significance level of 0.05.

Results

From the three treatments, the number of *Aa* bacterial colonies from the positive control tube TPC test, negative control, tube 4, tube 5, tube 6, and tube 7 were obtained as shown in Figures 2 and 3.

Table 1 shows that the MIC of okra fruit extract on *Aa* bacteria is on the sixth tube at a concentration of 3.125%, and the minimal bactericidal concentration (MBC) is on the fifth tube at a concentration of 6.25%.

Data obtained showed that they were normally distributed based on the Kolmogorov–Smirnov test normality test, then Levene homogeneity analysis test showed that data were homogeneous with $P = 0.215$ (>0.05) [Table 2]. The results of the research data were analyzed using one-way ANOVA statistical test [Table 2], the results showed that there was a significant difference ($P = 0.000$) between the control group compared to the treatment group giving okra fruit extract (*Abelmoschus esculentus*). While the statistical analysis using the Tukey HSD test showed that a significant difference occurred between the control group and the treatment group in the administration of okra (*A. esculentus*) fruit extracts at concentrations of 3.125%, and 1.565%. This means that there are significant differences in inhibiting or killing the *Aa* bacteria [Table 3].

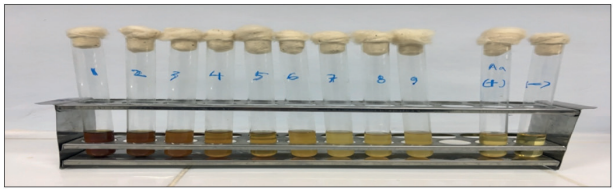

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Figure 1: Results of serial dilution of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria. The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration. Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract concentration. Tube 5 contains 6.25% okra extract concentration. Tube 6 contains 3.125% okra extract concentration. Tube 7 contains 1.563% okra extract concentration. Tube 8 contains 0.78% okra extract concentration. Tube 9 contains 0.39% okra extract concentration. The tube (+) is a positive control. Tube (−) is a negative control

Discussion

On the basis on the results of data analysis from the one-way ANOVA test in Table 1, *the P* value was found to be 0.000, indicating that if $P < 0.05$, it means that there is a significant difference between the control group and the treatment group. The results obtained indicate that the administration of natural okra (*A. esculentus*) extracts is effective in inhibiting or killing the *Aa* bacteria, which are predominant bacteria causing AP.

From previous studies it was said that phytochemical ingredients such as quercetin have antimicrobial activity against gram-positive and gram-negative bacteria.[9]

The effectiveness of the extract of okra fruit (*A. esculentus*) is caused by its content in the form of secondary metabolite components such as flavonoids, alkaloids, terpenoids, and quercetin.[6]

The antibacterial effect resulting from the extraction of okra against *Aa* is due to the presence of active substances that are soluble and contained in 70% ethanol solvent, which is a flavonoid and is a polar compound, which is generally soluble in polar solvents, namely phenols and quercetin.[10] During the extraction process of okra fruit (*Abelmoschus esculentus*) ethanol solvent is used because ethanol is a polar solvent that has a hydroxyl group (OH) that participates in the formation of hydrogen bonds which is the cause of the liquid is difficult to evaporate when compared with other organic compounds.[11]

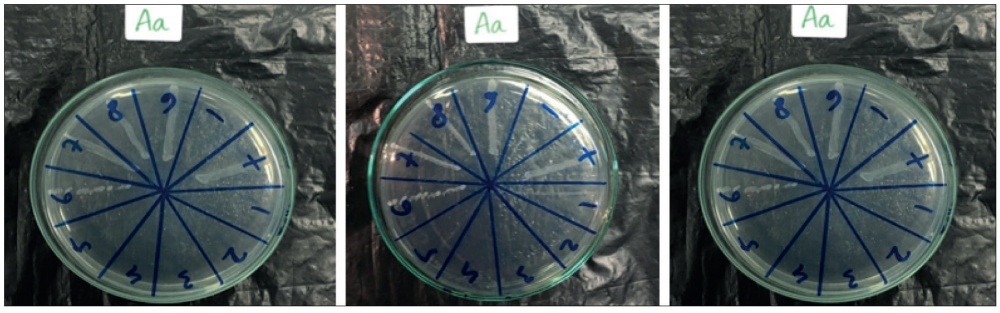

Figure 2: Results of scratches from 11 test tubes that showed the presence of *Aggregatibacter actinomycetemcomitans* bacteria growth in Mueller Hinton media from three replications

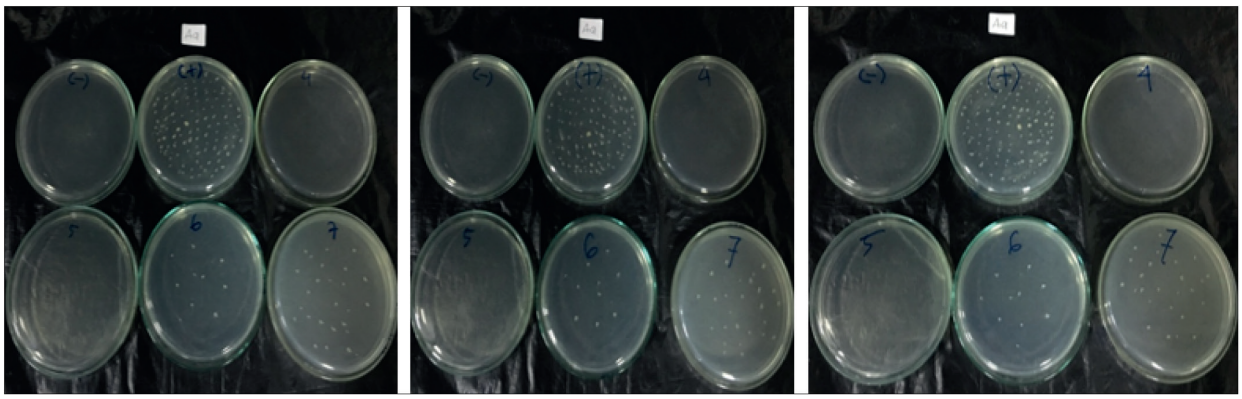

Figure 3: Total plate count test on Mueller Hinton media from positive control tube, negative control tube, number 4 tube, number 5 tube, number 6 tube, and number 7 tube from three replications

Table 1: Results of the number of *Aggregatibacter actinomycetemcomitans* bacterial colonies

Tube	Concentration of okra fruit extract	Number of *Aa* bacterial colonies (CFU/mL)		
		Treatment 1	Treatment 2	Treatment 3
4	12.5%	-	-	-
5	6.25%	-	-	-
6	3.125%	11	15	13
7	1.565%	25	28	26
(+)	100% + bacteria	116	126	120
(−)	100% without bacteria	-	-	-

Table 1 shows that the minimal inhibitory concentration of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria is on the sixth tube at a concentration of 3.125% and the minimal bactericidal concentration is on the fifth tube at a concentration of 6.25%

Table 2: One-way analysis of variance test for bacterial *Aggregatibacter actinomycetemcomitans* between groups

	Sum of squares	*df*	Mean square	*F*	Sig.
Between groups	20,668.667	2	10,334.333	979.042	0.000*
Within groups	63.333	6	10.556		
Total	20,732.000	8			

*Significant

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Table 3: Tukey honestly significant difference test for bacterial *Aggregatibacter actinomycetemcomitans* between concentration

Group	*N*	Subset for alpha = 0.05		
	1	2	3	1
kons.3.125%	3	13.0000		
kons.1.565%	3		26.3333	
Kontrolpos	3			120.6667
Sig.		1.000	1.000	1.000

Quercetin has many biological properties such as antioxidants, nerve protection, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, and anti-obesity.[12] It has been widely used in herbal medicine as traditional medicine for hundreds of years.[13]

The antibacterial potential of quercetin against the *Aa* bacteria is caused because quercetin has the ability to react to form complex components with metals such as Ag, Au, and Fe.[12] This is one reason that quercetin has potential antimicrobial activity.[14] Antibacterial activities of quercetin are mechanism against the cytoplasmic membrane of the bacteria, which is damaged through the perforation action of the quercetin. The inhibition of both energy metabolism and the synthesis of nucleic acids is another mechanism.[15] Flavonoids as antimicrobials, which are one of the active ingredients of okra fruit extract, have three mechanisms of action in killing microbes, the first possibility is to inhibit the synthesis of nucleic acids, the second is to inhibit the function of cell membranes, and the third is to inhibit the metabolism in bacterial cells, from all three aspects, flavonoids can cause damage to permeability in bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial deoxyribonucleic acid. The mechanism of action of flavonoids inhibits the function of cell membranes to form complex compounds with extracellular proteins that can damage bacterial cell membranes and is followed by the release of intracellular compounds.[16] Flavonoids have the ability to inhibit cell membrane function by interfering with the permeability of cell membranes and

inhibiting the binding of enzymes, such as ATPase and phospholipase. The correlation between antibacterial activity and membrane disorders supports the theory that flavonoids can show antibacterial activity by reducing the fluidity of bacterial cell membranes.

Therefore, the results showed that there was a significant decrease in the number of Aa colonies in the administration of okra fruit extract with a concentration of 3.125%, while in the administration with a concentration of 6.25% there was no growth of *Aa* bacteria. On the basis of the role of the flavonoid content of okra fruit extract as aforementioned, okra fruit extract had the power to kill *Aa* bacteria, which was shown by the MIC in the administration of 3.125%, whereas the MBC was at 6.25%.

The okra fruit extract effectively kills the *Aa* bacteria, which is the bacterium that causes AP as indicated by MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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ANALYSIS OF IL-10 ANTI-INFLAMMATORY CYTOKINES EXPRESSION IN SALIVA OF SEVERE EARLY CHILDHOOD CARIES

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Running title: IL-10 CYTOKINES EXPRESSION IN SALIVA OF CARIES

Abstract:

Objective: This study aimed to analyze the expression of IL-10 in children with severe early childhood caries and caries-free children.

Method: Saliva taken from preschool aged children (4 to 6 years) was divided into two groups, ie heavy caries group with dmft> 6 and caries free with dmft = 0. Salivary lymphocyte cells were obtained from participants by rinsing their oral cavities with 10 mL of sterile and 1.5% NaCl solution while they gargled without swallowing for 30 s before expectorating the resulting uid into a sterile glass – a procedure repeated four times. The collected solution was centrifuged (15 min at 450 g) at 4°C and the pellets then mixed with 2 mL of Roswell Park Memorial Institute medium. For expression test of IL-10, flow cytometry test was used.

Statistical test used: The results were listed as the mean ± standard deviation. All statistical analyses were performed using SPSS 20 (IBM, New York, USA). The statistical difference was analyzed by *one way anova*. Correlation analyses were performed using tukey HSD with $P < 0.05$ being considered to be significant.

Results: IL-10 expression in saliva of children with severe early childhood caries was 3.32±0.79. Whereas, in children with no caries was 4.04±0.65.

Conclusion: IL-10 expression in children with severe early childhood caries was significantly lower than in caries-free children.

Key-Words: Severe Early Childhood Caries, Il-10, Lymphocyte Cells

Key Messages

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen associated

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moleculer patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, abscesses

Introduction:

Caries in early childhood (ECC) is a multifactorial disease resulting from interactions of various factors, namely: cariogenic microbes, exposure to carbohydrate fermentation and various social variables. ECC is a condition of health abnormalities found in children living in socially disadvantaged communities, such as malnourished people with social and health inequalities.[1] ECC has an impact on general health, ranging from local pain, infections, abscesses, difficulty in chewing, malnutrition, indigestion, and insomnia.[2]

The body's immune system functions to defend the human body from foreign inventors. A compromised immune system can cause various diseases, such as infection, aging, allergies, various organ disorders and other diseases, such as cancer and auto immune deficiency syndrome (AIDS).[3] The role of the body's immune system is becoming increasingly important in understanding the mechanisms of disease prevention. The effective function of the body's immune system is to immediately eradicate the infectious agent from the body. This is done by an interactive system of actions, namely innate (very specific), fast but non-specific and adaptive immune system.[4] which function as a pathogenic killer[5], produce antibodies, and CD8 + T cell cytotoxicity[6].

Immunity in the oral cavity is a system that makes a balance by controlling various microbes in the oral cavity that are fluctuating due to external aggression. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system.

The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines which function to limit tissue damage and to maintain tissue homeostasis.[7] Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

Various preventions of dental caries had been carried out, for example by brushing teeth properly, fluoridating with topical applications, and making vaccines which until now have not shown the expected results.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of early detection of S-ECC.

Subjects and Methods:

Study Design:

This was an observational analytic study using children with severe early childhood caries and caries-free children as the objects of research with a cross sectional study design. Ethical clearance test at Universitas Airlangga, Faculty of Dental Medicine was done with Health Research Ethical Clearance Commission number of 209/HRECC. FODM/IX/2017.

Sampling Criteria:

eight children with S-ECC and eight free of caries were taken from the saliva of kindergarten children aged 4 to 6 years in the south Surabaya region which were previously divided into two groups.

Group one was children with who were diagnosed with severe early childhood caries (S-ECC) marked by decay exfoliation and filling (def-t>6). Whereas, group two was kindergarten children diagnosed with free caries marked with def-t = 0.

5ml saliva taken from the kindergarten children with SECC and caries-free . Sampling was carried out by researchers and trained personnel using protocol standards. Subjects might not eat, drink, chew gum, or brush their teeth for 60 minutes before sampling. Furthermore, samples were frozen at -80oC for analysis.

Observational parameters:

Analysis of IL-10 expression was determined using flow cytometry, according to Luthfi *et al* (2019)[10a]. 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 optimal concentration of mAbs is determined for each mAb with titration. Flow cytometry simultaneously measures and analyzes the physical properties of particles such as cells because it flows through the flow of fluid through a beam of light. The nature of scattering cell light can be used to analyze changes in size, granularity, internal complexity and relative fluorescence intensity. Flow cytometry analysis was performed to determine directly the pattern of lymphocyte immunomodulation, using conjugated monoclonal antibodies (mAbs).

Statistical Analysis:

The results were listed as the mean ± standard deviation. All statistical analyses were performed using SPSS 20 (IBM, New York, USA). The statistical difference was analyzed by t Test.

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Inclusion and exclusion criteria
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Results:

Data from the results of the study before the test using the t test, conducted tests of normality and homogeneity using the Shapiro-Wilk test. From the results of these tests indicate a value of P> 0.05 which means that all data are normally distributed and homogeneous. Normality test using Shapiro Wilk shows) data with normal distribution with p = 0.844 (p> 0.05, while Levene test results show homogeneous data with p = 0.726 (p> 0.05).

The data obtained showed that the average IL-10 in the caries free group was higher than the S-ECC group, but the difference was not statistically significant between caries free and S-ECC

Discussion:

The results of the study showed that there was expression of IL-10 decrease in children with S-ECC compared to that in caries-free children. This was probably due to the S-ECC patients responding more antigens in the form of S. mutans bacteria which were relatively high in numbers compared to that in children with free caries.[10b]

An antigen structure called Pathogen Associated Molecular Pattern (PAMPs), which will be recognized by Pattern Recognition Receptors (PRRs), namely Toll-like receptors (TLRs), is very important for triggering the effector phase of the innate immune response.[11] TLR2 and TLR4 involved in the introduction of Gram-positive and Gram-negative bacteria that have been detected in odontoblast cell membranes in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through the dentinal tubules during carious infection.[12] One of the main consequences of TLR activation is an increase in the efficacy of innate immunity, including antimicrobial agents and proinflammatory cytokines and chemokines that recruite and activate immune cells.[13] One of the main consequences of TLR activation is increased efficacy of innate immunity, including antimicrobial agents and proinflammatory cytokines and chemokines that recruit and activate immune cells.[13] This causes the S-ECC saliva to increase prolonged inflammatory cytokines, IFN-γ expression increase.[14] which eventually can cause tissue damage that affects health in general, starting from local pain, infection, abscess, difficulty in chewing, malnutrition, indigestion, and sleeping difficulty.[15].

Based on the results of this study, high expression of proinflammatory cytokines in S-ECC should be balanced by the immune host system by producing anti-inflammatory cytokines, IL-10. As a response to pathogenic microbes, the body's adaptive immune system develops effector cells that function to prevent these threats, namely CD4 + memory T cells which serve as a protective against bacterial infections.[16] CD4 + cells participate in responding to secondary infections that have the potential as anti-pathogens [5] producing antibodies and CD8 + T-cell cytotoxicity.[6] However, this did not occur

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in S-ECC so IL-10 expression in S-ECC saliva was lower than that in caries-free children. This was probably due to the role of the immune system in S-ECC which was not as good as that in caries-free children.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than caries-free

Acknowledgement: Department of Oral Biology, Faculty of Dentistry, Universitas Airlangga

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Conflict of interest:

There is no conflict of interest in this research

Author 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

Aqsa Sjuhada Oki: Study concept, clinical studies, experimental studies, data analysis, manuscript review

Retno Indrawati: data interpretation, Statistical analysis, manuscript preparation, manuscript editing,

Priyawan Rachmadi: Statistical analysis, manuscript preparation, manuscript editing,

Muhaimin Rifa'i: manuscript editing, manuscript review

Patient declaration of consent:

Before saliva sampling from children aged 4 to 6 years, parents of the sample had agreed to signed a written informed consent.

Data Availability statement:

Dataset can be made available after embargo period due to commercial restrictions

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Abbreviations

ECC : Early childhood caries
S-ECC : Severe early childhood caries
IL-10 : Interleukin-10
PRRs : Pathogen recognition receptors
TLR : Toll like receptors
PAMPs : Pathogen associated moleculer patterns
CD8 : Cluster differentiation 8
CD4 : Cluster differentiation 4
def-t : Decay exfoliation and filling
FITC : Fluorescein isothiocyanate
PE : Phycoerythrin
APC : Allophycocyanin
PerCP : Peridinin chlorophyll protein

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Table 1. Normality test uses Shapiro Wilk IL-10 expression from S-ECC and caries free

variable	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
IL10	Statistic	Df	Sig.	Statistic	Df	Sig.

	,143	16	,200*	,970	16	,844

Table 2. Mean and standard deviation of IL-10 expression in S-ECC and caries free analyzed by flow cytometry test which was tested using independent t Test

Group	n	IL-10 Expression (%)	
		Mean ± SD	P
Caries Free	8	4,04 ± 0,89	0,11
S-ECC	8	3,32 ± 0,76	

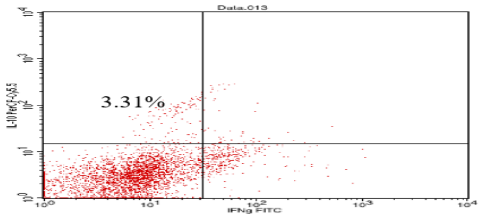

Figure 1. Expression of IL-10 from S-ECC salivary after analyzed by Flow Cytometry test.

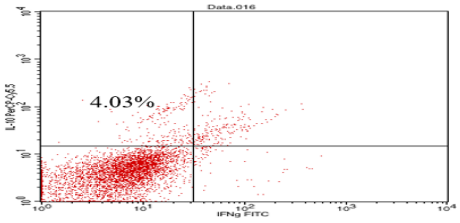

Figure 2. Expression of IL-10 from caries free salivary (B) after analyzed by Flow Cytometry test.

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A manuscript has been submitted to our journal Journal of International Oral Health by Udijanto Tedjosasongko titled 'Analysis of IL-10 Anti-Inflammatory Cytokines Expression in Saliva of Children with Severe Early Childhood Caries and Caries-Free Children'. A copy of the acknowledgment mail is attached here with for your reference.

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Journal of International Ora l Health has received your manuscript entitled "Analysis of IL-10 Anti-Inflammatory Cytokines Expression in Saliva of Children with Severe Early Childhood Caries and Caries-Free Children" for consideration for publication. The reference number for this manuscript is "jioh_293_18". Kindly quote this in correspondence related to this manuscript.

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Original Research

Analysis of Interleukin-10 Anti-inflammatory Cytokines in Salivary Lymphocyte Surface: A cross Sectional Study

Retno Indrawati[1], Muhammad Luthfi[1], Aqsa S. Oki[1], Yuliati[1], Agung Sosiawan[2], Priyawan Rachmadi[3], Muhaimin Rifai[4]

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Abstract

Aim: To analyze the expression of interleukin-10 (IL-10) in children with severe early childhood caries (S-ECC) and caries-free children. **Materials and Methods:** This was an observational analytic pilot study performed on children with social factors-ECC (S-ECC), and caries-free children as the objects of research with a cross-sectional study design. Saliva of children aged 4–6 years from the group of caries children in severe and caries-free early childhood was taken. Samples were taken by rinsing with 1.5% sterile NaCl for 30 s and then accommodated in a sterile tube, to get a 40 mL sample from the aforementioned procedure repeated four times. Flow-cytometry test was used to analyze the IL-10 expression. The results of the study were analyzed using the normality test using Shapiro–Wilk, then continued with *t* test using the Statistical Package for the Social Sciences (SPSS) software program, version 20.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by independent *t* test to see the difference between caries-free children and S-ECC. **Results:** The expression of IL-10 in the saliva of children with severe ECC was 3.32 ± 0.79; meanwhile, in caries-free children it was 4.04 ± 0.65. **Conclusion:** The IL-10 expression in children with severe ECC was significantly lower than that of in caries-free children.

Keywords: Interleukin-10 Anti-inflammatory Cytokines, Lymphocyte Cells, Severe Early Childhood Caries

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Introduction

Dental caries is a multifactorial disease due to various factors, namely cariogenic microbes, carbohydrates, and social factors, whereas early childhood caries (ECC) is often found in children with low-socioeconomic conditions.[1] Several studies have recognized the importance of infection of *Streptococci mutans*.[2]

The immune system is a very varied compilation of cells, consisting of two parts of the immune system, namely innate and adaptive. The innate and adaptive immune systems are interrelated, and recognition by innate immune systems can cause the activation of the adaptive immune response.[3] The innate immune system is the first line of host defense against pathogens and recognizes molecules repeatedly against pathogens, which are called pathogen-related molecular patterns through germline-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[4]

The components that regulate the immune system, such as an immune regulator cells and regulating cytokines, both natural and acquired as induced by an antigen, plays an important role in controlling various immune responses, both physiological and pathological. Local and systemic interleukin-10 (IL-10) responses have been shown to have pathophysiological relevance in several diseases such as malignancy, infectious diseases, autoimmune diseases, and atopic disorders.[5] Because of

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this, IL-10 can activate signal transducer and activator of transcription 3 (STAT3) in macrophages and T cells to restore and respond to the presence of pro-inflammatory cytokines.[6]

Immunity in the oral cavity's immune system has an important role that is balancing the amount of microbes in the mouth. The microbial activity in oral cavity can be fluctuatives, due to pathogen situations. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system. The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine, which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines that function to limit tissue damage and to maintain tissue homeostasis.[7] IL-10 is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

For dental caries preventions, many efforts had been carries out. The government and supporting health organization ran some prevention programs, such as dental counseling to community. Most of the programs targeted children, pregnant woman, and elderly. They teach how to brush teeth properly, dietary that good for dental health, prevention treatment that can be applied for children, and vaccines.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of social factors-ECC (S-ECC).

Materials and Methods

This was an observational analytic study using children with S-ECC and caries-free children as the objects of research with a cross-sectional study design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 209/HRECC.FODM/IX/2017).

Sixteen children with S-ECC and caries-free were taken from preschool children aged 4–6 years, in the southern Surabaya region, which had previously been divided into two groups.

Group one were children with a diagnosis of S-ECC characterized by decay, extraction, and filling (def-t > 6), whereas, the second group were preschool children who were diagnosed with free caries marked with def-t = 0.

5 mL saliva is taken from preschool children with S-ECC and caries free. Sampling was carried out by researchers and trained research assistants using standard protocols. Subjects were asked not to consume food and drink, or brush their teeth for 60 min before the study was conducted. The samples obtained were stored at −80°C for analysis. IL-10 expression was analyzed using flow cytometry, according to Luthfi *et al.*[10]

Statistical analysis: The data were analyzed by independent t test to see the difference between caries free and S-ECC.

Results

Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the SPSS Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

The data obtained indicate that the average IL-10 in the caries-free group was higher than the S-ECC group, but the difference was not statistically significant between caries-free and S-ECC.

Discussion

From the data obtained from the research results then performed statistical calculations. Before testing and analysis between S-ECC groups and caries free, the normality test was done in each group using the Shapiro-Wilk test which obtained the result that the value of p > α = 0.05 is P = 0.844 which means that the data are normally distributed, as shown in Tables 1 and 2 is the result of statistical analysis between S-ECC and caries free using independent t test, the value of p = 0.11 means that there are differences in expression even though statistically it is not showed significant results because the value of

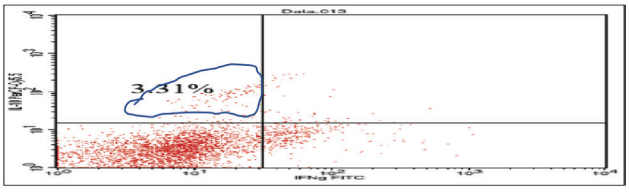

Figure 1: Expression of interleukin-10 (3.31%) from severe early childhood caries salivary after analyzed by flow-cytometry test

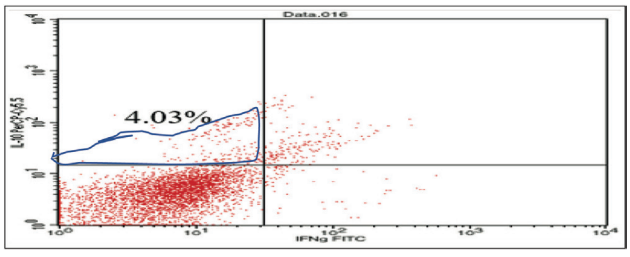

Figure 2: Expression of interleukin-10 (4.03%) from caries free salivary after analyzed by flow-cytometry test

Table 1: Normality test using Shapiro–Wilk interleukin-10 expression from severe early childhood caries and caries free

Variable	Kolmogorov–Smirnov			Shapiro–Wilk		
IL-10	Statistic	Df	Sig.	Statistic	Df	Sig.
	143	16	200	970	16	844

IL-10 = interleukin-10, Df = degrees of freedom

Table 2: Mean and standard deviation of interleukin-10 expression in severe early childhood caries and caries free analyzed by flow-cytometry test, which was tested using independent *t* test

Group	*n*	IL-10 expression (%)	
		Mean ± SD	*P*
Caries free	8	4.04 ± 0.89	0.11
S-ECC	8	3.32 ± 0.76	

S-ECC = severe early childhood caries, SD = standard deviation, IL-10 = interleukin-10

p> 0.05. on the surface of lymphocyte cells in saliva the S-ECC group that expressed IL-10 (3.31%) was less than that of the caries free group that expressed IL-10 (4.03%) this will be clarified in Figures 1 and 2.

Based on Figure 1 which is the result of examination using flow cytometry test shows that lymphocytes in saliva severe early childhood caries express IL-10 of 3.31%, while in Figure 2 which is the result of examination using flow cytometry test shows that lymphocytes in caries-free children express IL-10 at 4.03%. This shows that the S-ECC saliva is less specialized in proinflammatory cytokines and conversely expresses inflammatory cytokines which results in chronic inflammation. The occurrence of chronic inflammation is caused because innate immunity in S-ECC is not as good as in free caries so innate immunity is not able to fight the pathogens that cause dental caries.

IL-10 is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen-associated molecular patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, and abscesses.

The results showed the occurrence of decreased IL-10 expression in preschool children with S-ECC compared with in caries-free children. This may be preschool children with S-ECC responding to more antigens in the form of *S. mutans* bacteria, which are relatively high in number compared to children with free caries.[11]

Antigen structures called PAMPs, which will be recognized by PRRs, namely TLRs, are very important to trigger the effect or phase of the innate immune response.[12] TLR2 and TLR4 involved in the introduction of gram-positive and gram-negative bacteria that have been detected in the odontoblast cell membrane in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through dentinal tubules during carious infection.[13]

One of the main consequences of TLR activation is an increase in innate immune efficacy, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] One of the main consequences of TLR activation is an increase in the effectiveness of innate immunity, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] This causes a prolonged increase in inflammatory cytokines in S-ECC, increasing IFN-γ increase expression.[15] which can ultimately cause oral cavity tissue damage that affects general health, ranging from local pain, infections, abscesses, difficulty chewing, malnutrition, indigestion, and trouble sleeping.[16]

Study shows that an increase in pro-inflammatory cytokines occurs in S-ECC; this must be balanced by the host immune system by producing anti-inflammatory cytokines, IL-10. Cluster differentiation 4 ($CD4^+$) memory T cells are developed in response to pathogenic microbes. $CD4^+$ memory T cells prevent the body from fighting pathogens.[17] $CD4^+$ cells also respond as antipathogens,[18] which produce antibodies and cytotoxicity of cluster differentiation 8 ($CD8^+$) T cells,[19] but this does not occur in S-ECC so IL-10 expression in S-ECC saliva is lower than in caries-free children. This study requires larger sample size to evaluate the expression in different age groups and populations.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

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Republic of Indonesia for the grant funding provided for this research.

Conflicts of interest

There are no conflicts of interest.

Authors contributions

- Muhammad Luthfi: Study conception, study design, intelectual content, literature research, data acquisition, data analysis, manuscript review, guarantor.
- Aqsa Sjuhada Oki: Study concept, clinical studies, experimental studies
- Yuliati: Study concept, clinical studies, experimental studies
- Agung Sosiawan: Data analysis, manuscript review
- Retno Indrawati: data interpretation, Statistical analysis, manuscript preparation, manuscript editing,
- Priyawan Rachmadi: Statistical analysis, manuscript preparation, manuscript editing,
- Muhaimin Rifa'i: manuscript editing, manuscript review

Ethical policy and Institutional Review board statement

Ethical clearance test at Universitas Airlangga, Faculty of Dental Medicine was done with Health Research Ethical Clearance Commission number of 209/HRECC. FODM/IX/2017

Patient declaration of consent

Before saliva sampling from children aged 4 to 6 years, parents of the sample had agreed to signed a written informed consent.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions

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