Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries-Free in Surabaya

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EXTENDED ABSTRACT

Analysis of Lymphocyte Cell Proliferation and IFN-γ Expression In Saliva of Severe Early Childhood Caries and Caries-Free in Surabaya

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SUMMARY

Dental caries is a chronic, multifactorial disease and occurs by shifting of biofilm. Shifting of biofilm flora caused by poor oral hygiene, genetic factors, and long-term immune changes lead to an increase in *Streptococcus mutans* which results in decreased pH and demineralization on tooth surfaces. The body's immune system serves to defend from foreign invaders. IFN-γ and lymphocytes are innate and adaptive immunity components that are responsible for the occurrence of disease. The role of the immune system becomes 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. The objective of this study was to analyze the expression of IFN-γ and salivary cell lymphocyte proliferation as an early detection marker of severe early childhood caries. 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, for lymphocyte cell proliferation test using MTT assay and for expression test of IFN-γ using flow cytometry test. There are differences in lymphocyte cell proliferation and IFN-γ expression in the saliva of severe early childhood (S-ECC) caries and caries-free. The increase in lymphocyte proliferation and IFN-γ expression up to 6 hours incubation can be used as the indicator of early detection marker of severe early childhood caries.

Keywords: Lymphocyte Cell, IFN-γ, Severe early childhood caries (S-ECC), Early detection marker

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INTRODUCTION

In Indonesia, the prevalence of caries in children aged 3-5 years continues to increase. In 2001, caries prevalence in children aged 3-5 years in DKI Jakarta was 81.2% [1]. In Surabaya, the prevalence of Early Childhood Caries (ECC) in groups of children 6 months-3 years was 30.8%; while the prevalence of Severe Early Childhood Caries (S-ECC) was 29.2% [2]. The process of caries occurrence is the result of a gradual and consistent shift in the balance of demineralization and remineralization of tooth enamel directly affected by S.mutans is one of the most important etiologic agents as the cause of dental caries [3].

Immune system of the body serves to defend the human body from foreign inviders.Immune system can cause various diseases such as infection, aging, allergies, disorders of various organs and other diseases such as cancer and auto immune deficiency syndrome (AIDS) [4]. The role of the Immune system of the body becomes increasingly important in conceiving the mechanisms of disease prevention. The effective function of the body immune system is to immediately eradicate the infectious agent from the body. This is done by mutually interactive system actions, ie innate (very specific) fast but non-specific and adaptive immune system [5].

In response to pathogenic microbes, the adaptive immune system of the body develops effector cells that function to prevent such threats, ie: CD4+ T cell memory that serves as a protective against bacterial infections [6].CD4 + cells participate in response to secondary infections that are potentially anti pathogenic [7]. Producing antibodies, and CD8 + T cell cytotoxicity [8].

Immune in the oral cavity is a system that makes the balance by controlling various microbes contained in the oral cavity that fluctuate due to external aggression. Various dental caries prevention has been done, for example by correct brushing, fluoridation with topical application, and vaccine making which has not yet shown the expected results [9]. Therefore this research was aimed to identify the risk factor of dental caries in

the form of immune system both innate and adaptive immunity which one of its function is to eliminate pathogen that attacking the host. It is very importance to analyze the proliferation of lymphocytes and IFN-γ expression in saliva and caries-free as early detection marker of severe early childhood caries.

MATERIALS AND METHODS

Lymphocyte Isolation

Salivary lymphocyte was obtained by instructing the subjects to gargle with 10 ml of 1.5% sterile NaCl solution while garglingfor 30 seconds, then exporiated intoa sterile glass. This procedure was repeated 4 times. Next amples was centrifuged at 450g for 15 minutes, at 4°C. The centrifugation pellets were then mixed with 2 ml of medium RPMI, and then the samples are then filtered sequentially with 20 and 11 µm nylon filters [10]. The resulted in a cell suspension and then was calculated using a hemocytometer.

The same volume of cell suspension and 0.2% blue tripan dye was mixed in Eppendorf tube and vortexed. The same suspension aliquot (20 µl) was added to both chamber haemocytometers and observed under a microscope (10X objective). The mixture was drawn digrid with capillary action. The cells were counted in an area of 16 squares which was equal to the number of cells x104 / ml. Only translucent cells are counted in the box. The number of cells per ml was calculated using the following formula:

Cell / ml = average number of cells per primary square x104 x dilution factor

Lymphocyte Proliferation Test

MTT assay is the standard used to measure cell viability. This is colorimetric test that measures cell proliferation. MTT assay is based on the reduction of the yellow tetrazolium compound, 3- (4,5-dimethy thiazol-2) -2,5-diphenyl tetrazolium bromide (MTT) by mitochondria succinic dehydrogenase. MTT entered the cell and entered the mitochondria and was reduced to a color solution (dark purple) formazan crystal. These cells were then dissolved with organic solvents and released, formazane reagents were measured using a spectrophotometer [11]. The optimum wavelength for absorbance was 570 nm. The research results were analyzed by plotting the number of cells against absorbance, followed by changes in quantization of cell proliferation. The tetrazolium reduction rate was proportional to the rate of cell proliferation.

$$\% \text{ Cell Proliferation} = \frac{\text{Average absorbance of treated cells}}{\text{Average absorbance of untreated cells}} \times 100$$

IFN-γ Expression Analysis with Flow Cytometry Principle

Analysis of IFN-γ expression was determined using flow cytometry, according to manufactur Fluoresceinisothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Peridinin chlorophyll protein (PerCP), PerCP-Cy5.5-conjugated monoclonal antibodies (mAbs) (Becton Dickinson San Jose, CA, USA). The optimal concentration of mAbswas determined for each mAb by titration. Flow cytometry simultaneously measures and analyzes the physical properties of particles such as cells as they flow through the flow of fluid through a beam of light. The nature of scatter cell light can be used to analyze changes in size, granularity, internal complexity and relative fluorescence intensity. Flow cytometric analysis was performed to directly determine the immunomodulation pattern of lymphocytes, using conjugated monoclonal antibodies (mAbs).

This research was approved by The Ethics Committee of the Faculty of Dental Medicine Universitas Airlangga with an ethical certificate number: 209/HRECC.FODM/IX/2017.

RESULTS

The result of the research in caries freeshowed that there was an increase of lymphocyte cell proliferation at incubation until 4hours then decrease of lymphocyte proliferation at 6 hours incubation, whereas in the S-ECC group the decrease of lymphocyte cell proliferation in 4 hours incubation increased again after 6 hours incubation as seen in Figure 1.

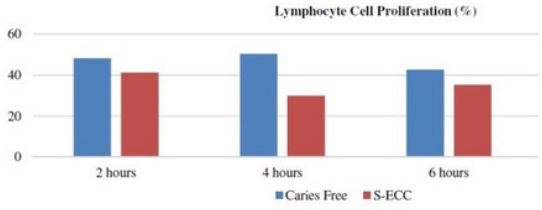

Figure 1: Lymphocyte proliferation after incubation of 2 hours, 4 hours and 6 hours on caries free and S-ECC (n=8)

The results of ttest statistic analysis in the caries-free group showed that there was an increase in IF-γ expression in 4 hours incubation but decreased at 6 hours incubation, but in the S-ECC group increased IFN-γ expression up to 6 hours incubation as seen in Figure 2.

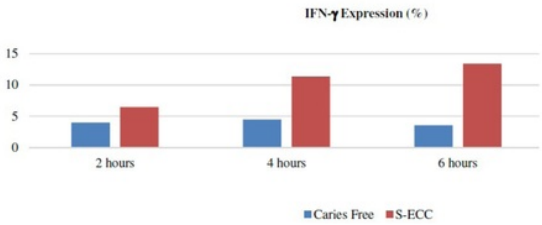

Figure 2: The average graph of IFN-γ expression on the surface of saliva netrofil after incubation of 2 hours, 4 hours and 6 hours in free caries and S-ECC (n=8)

DISCUSSION AND CONCLUSION

Dental caries occur by microbiological shifts in biofilms. Shifting of biofilm flora caused by poor oral hygiene, genetic factors, and long-term immune changes leads to an increase in Streptococcus mutans which results in decreased pH and demineralization on tooth surfaces. Immune system of the body serves to defend the human body from foreign inviders.

Soluble mediators of the innate response provide a network of signals to organize molecular and cellular response to infection, including direct and immediate antimicrobial activity. Innate response provides a signal network to regulate molecular and cellular responses immediately close to infection, including direct and immediate antimicrobial activity [12].

IFN-γ is a proinflammatory cytokine that supports cellular immunity secreted from innate and adaptive immune cells due to the effects of cytokines such as IL-12 and IL-18. IFN-γ was first described for its antiviral activity but is currently known to protect against certain types of microbial infections and mice lacking IFN-γ or IFN-receptors can cause infections that easily caused by microbes.

IFN-γ causes cytotoxic T cell responses (CD8) and up-regulation of presenting Cell antigen (APC) class II to enhance the activation of TH4-cell CD4 antigens. Furthermore, it encourages narive CD4 + T-cells to commit to the TH1 phenotype. In addition, IFN-γ may inhibit cell growth and induce apoptosis to reduce the TH2 population [13]. It is possible that the lymphocyte cell proliferation and IFN-γ expression increase in S-ECC up to 6 hours incubation time is compared to free caries. Conclusion, increase in lymphocyte proliferation and IFN-γ expression up to 6 hours incubation can be used as the indicator of early detection marker of severe early childhood caries.

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PAGE 1

PAGE 2

PAGE 3