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Innate Immunity Signatures of Early Childhood Caries (ECC) And Severe Early Childhood Caries (S-ECC)

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Caries that affects children under the age of 6 is known asearly childhood caries (ECC). According to the American Academy of Pediatric Dentistry (AAPD), ECC is defined as the presence of one or more teeth with caries (cavities or no cavities), missing teeth due to caries or the presence of restoration deciduous teeth in children under 71 months. If it occurred on the smooth surface of the tooth in children under 3 years of age, the disease is classified as severe early childhood caries (S-ECC). *Streptococcusmutans* plays important role in the etiology of caries of preschool children, which is a contagious pathological entity.The pathogenisan acidogenic and aciduricbacteria.The occurrence of caries is a complex process of interaction between host factors (teeth), microorganisms, substrates (carbohydrates) and the environment, including saliva. This mini review aims to summarizecurrent findings on several markers of innate immune system related to ECC and S-ECC namely lysozyme, salivarylactoferrin, IL-8, CD36 of salivaryneutrophils, and sCD14.

Keywords: ECC, S-ECC, Innate immune system, Biomarkers.

Introducing ECC & S-ECC

Caries is a chronic infectious disease that affects the hard tissues of the teeth and can occur in adults or children. Caries that affects children under the age of 6 is called early childhood caries (ECC). According to the American Academy ofPediatric Dentistry (AAPD)[1], ECC is defined as the presence of one or more teeth with caries (cavities or no cavities), missing teeth due to caries or the presence ofrestoration of deciduous teeth in children under 71 months or 6 years. If the children under 3 years of age are experiencing caries on the smooth surface of the teeth, it indicates severe early childhood caries (S-ECC). The ECC starts with a white spot on the deciduous / upper maxillary incisors along the gingival margin, and if caries continues it will damage the entire crown of the tooth.[2]

The prevalence of ECC in developing countries reaches 85% in the weak economic group.[3]The American Academy of Pediatric Dentistry (AAPD) found that 70% of children aged 2-5 years have caries. In Indonesia the prevalence of caries in 5-year-olds is 67.3%, with a caries experience rate of e" 6, this is a severe category of ECC.[4]

The factors responsible in ECC are supportive host, fermented carbohydrate diets, dental plaques, and the number of cariogenic microbes, such as *Streptococcusmutans* and *Lactobacillus*.[5]*S.mutans*in an essentialcausative agent of caries among preschoolers.This species is both acidogenic and aciduric, with the resulting caries shown to be contagious.[6] Initial acquisition of *S. mutans* is the key to caries disease, in which acquisition can occur through vertical and horizontal transmission.[7]The caries process occurs when the activity of bacterial (*Streptococcus mutans*) enzymes in the oralfermentated carbohydrate and produce acids, it will cause demineralization of the tooth surface.[8] If the demineralization process is more often or extensive than the remineralization process, caries occurs.[3]Recent study on a geriatric population found that *S. mutans* is also intriguingly related to higher risk of suffering stroke and dementia.[9]Whether acquisition of this bacteria is associated to increased risk of having reduced cognitive performancein children, other than causing caries, deserves further attention.

Several studies have been carried out to find out the markers related to caries. Mass *et al*. found an association between low levels of lysozyme and decreasing in the number of *S.mutans*.[9]Klissia *et al*. found the relationship of lysozyme todmft score, and lactoferrinversus the number of restored teeth in school children. The level of lactoferrin and lysozyme may be usedto evaluate caries status.[10]In view of these findings, we aimed to presentcurrentknowledgeonseveral biomarkers associated with ECC and S-ECC, especially from the point of view of innate immune system.

Lysozyme

Lysozyme is a salivary protein that plays an important role in innate immunity. This 14 kDa basic protein is found in a vast array of body fluids such as plasma/serum, tears, amniotic fluid, saliva, urine, bile and CSF. This enzyme has the ability to act as an antibacterial agent. In the oral cavity, lysozyme is secreted from the major and minor salivary glands, gingival crevicularfluid (GCF), and salivary leukocytes. Lysozyme exhibits the activity of muramidase, whichhydrolyzesâ-(1-4) bonds between N-asetylmuramide acid and N-asetylglucosamineofbacterial cell wall peptidoglycan, thus degrading bacterial wall integrity, resulting in hypo-osmolarization and bactericidal effect. As a strong cationic protein, lysozyme is a medium of aggregation and attachment of bacteria to activate the autolysin of bacteria, which also damages bacterial cell walls.[11,12]

Octiara *et al*. found that lysozyme is a promising biomarker of ECC. Higher lysozyme level was found in caries-free controls than that of ECC groups.[13] Rather than relying solely to salivary lysozyme level measurement, it is suggested that studying the lysozyme activity is also crucial because cariostatic activity of lysozyme is a resultant of its salivary concentration and status of activation.[14]Currently, clinical study that investigates both lysozyme level and activity, and their correlations with clinical outcomes (dmft scores) in ECC and S-ECC patients is lacking.

Lactoferrin

Lactoferrin is a salivary protein that plays an important role in innate immunity (non-specific body defense system). Lactoferin is a 75kDa salivary protein that has a high affinity for the iron binding glycoprotein. The cariopreventive role of this molecule is due to its effect in attracting iron (iron chelation) from bacteria.[11]Epithelial cells in the mucosa and neutrophils are known as the endogenous producers of this protein.[15]Lactoferrin is present in saliva, tears and secondary granule of leukocytes. Intriguingly, iron-free lactoferrin (apolactoferrin) has also antibacterial ability through direct binding of lactoferrin to bacteria and agglutination of *S.mutans*, thereby facilitating removal of agglutinated bacteria through mechanical action of salivary ingestion. *In vitro*,lactoferin has been shown to exhibit anti-inflammatory and antimicrobial effects.[15,16]

Studies on the association of lactoferrin and salivary lysozyme that are not stimulated on ECC showedthatthe levels oflactoferrin and lysozymeare higherin caries-free individuals than ECC-positive individuals.[16] The levels of lactoferrin and lysozyme decrease significantly after 3 months of comprehensive dental treatment of ECC patients.[17]However, other study have shown the opposite, i.e.,theupregulation of salivary lysozyme levels along with the increased lysozyme activity in preschool children with S-ECC compared with caries-free groups.The inconsistency between studies may be the resultant

of a number of factors such as different ways applied for lysozyme quantification, dentition status (deciduous versus permanent), and the temporal regulation of lysozyme level (i.e., age-related changes).[18]

Interleukin 8 (IL-8)

Neutrophils in saliva is the first defense of the immune cell against microbial pathogens, it can recognize bound surfaces or free molecules secreted by bacteria including *S mutans* such as peptidoglycan, lipoprotein, lipoteichoic acid (LTA), lipopolysaccharide (LPS), CpG-containing DNA, and flagelin. These pathogenic molecules, collectively known as pathogen associated molecular patterns (PAMPs), interact directly with a number of pathogen recognition receptors (PRRs) expressed on the cell surface, including toll-like receptors (TLRs).[19]

Interleukin-8 (IL-8) is a member of CXC family chemokine. IL-8 is a key mediator in the migration of neutrophils to areas of inflammation and infection.[20,21]Interleukin-8 (IL-8) is synthesized by polymorphonuclear leukocytes (PMNs), with lactoferrin as an endogenous stimulator for the secretion.[22] If there is stimulation of microbial groups, G-CSF or GM-CSF, tumor necrosis factor-á (TNF-á) or type I and II interferon in the inflamed tissue, activated neutrophils will produce IL-8.[23]IL-8 is secreted by macrophages and endothelial cells as neutrophil attracti9 chemicals, leadthe infiltration of neutrophils. *In vitro* IL-8 results in the recruitment of neutrophils and increases the release of lysosomal enzymes against bacteria.[24,25]

Luthfi *et al*. showed that IL-8 expression was lower in S-ECC (early childhood caries) than caries-free early childhood. The lower expression of IL-8 in S-ECC salivary neutrophils causes the killing of *S. mutans* to be less effective because the oxidative activity of NADPH was low.The degranulation process is also becoming less active so that the killing in the phagosome decreased, thus the cariogenesis is continued. The reduced expres1n of IL-8 in salivary neutrophils may be one of the causes of the increasing number of *S.mutans* in S-ECC.[26]

AZurophilic Granules (CD63) In Salivary neutrophil

Neutrophils are phagocytic cells in the first line of defense against pathogenic bacteria. Primary granules (azurophilic granules) in salivary neutrophils contain antimicrobial molecules, such as defensin like human neutrophil peptide 1-3 (HNP1-3), elastase, cathepsin G and proteinase 3, and CD63. These granules are related to the phagocytosis tool which releases its contents in the phagosome which causes microbes are phagocytozed. Gram-positive and gram-negative microbes, including *S mutans*, are the main bacteria that cause dental caries.[27]Lutfi's study showed that CD63 neutrophil salivary expression decreased in S-ECC.[25]

The low neutrophil salivary CD63 expression on S-ECC is caused by S *mutans* which

Table 1. Summary on Biomarkers Related to ECC and S-ECC

Markers	Conditions	Related Molecular/ Cellular Events	Outcomes	References
Lysozyme	High	High sIgA level	Associated to ECC-free phenotype and lower dmft score	13
Lactoferrin	High	Anti-inflammatory action (*in vitro*)	Associated to caries-free phenotype	12, 15
IL-8	Low	Reduced neutrophil recuitment	Associated to S-ECC severity or progression	20, 23, 24,
CD63 in salivary neutrophils	Low	Higher level of *S. mutans*; Elastase and cathepsin G deficiency	Associated to S-ECC severity	25, 26, 28
sCD14	Undetectable or Low	Formation of stable complex with bacterial LPS	Associated to ECC phenotype	31, 32, 34

Table 2. Genes,Chromosomal Loci, and Local Sourcesof ECC- and S-ECC Potential Biomarkers

Markers	Encoding-genes	Cytogenetic Loci	Oral Sources	References
Lysozyme	*LYZ* (*LYZF1*, *LZM*)	12q15	Salivary glands, salivary neutrophils	11, 12, 36
Lactoferrin	*LTF* (*LF*)	3p21.3	Mucosal epithelial cells and neutrophils	15,37, 38
IL-8	*CXCL8*	4q13.3	PMN leukocytes	22, 39
CD63	*CD63* (*MLA1*)	12q13.2	PMN leukocytes	40, 41
CD14	*CD14*	5q31.3	Monocytes (secreted upon activation)	42, 43

has been internalized by neutrophils through the process of phagocytosis mediated by FcáR (CD89) or CR1 (CD35) which can develop into three defense system strategies to avoid intracellular killing, first, leaving the phagosome, second, the combined blocking of phagosome-lysosomes, and third, using a mechanism that allows survival in phagolysosomes.[28] Low neutrophil salivary CD63 on S-ECC is also caused by protein elastase and cathepsin G deficiency.[29]

Lack of active neutrophils will produce less extracellular traps (NETs) that work to kill extracellular microbes because NETs contain lactoferrin, cathepsin and enzymes which are highly toxic for microbes, in addition to facilitating the phagocytosis process.[30]Decreased expression of salivary neutrophil CD63 is found to be associated with higher *S.mutans* levels in S-ECCcases.[26]

Soluble CD14

Other potentially useful salivary biomarker of early carious lesions in children is soluble or saliva CD14 (sCD14).One study finds that this molecule is undetectable through Western blotting in the saliva of early childhood caries patients, meanwhile the opposite state does exist in normal control. Following dental treatment against the lesions, sCD14 is detectable in the saliva.[31]Similar pattern of relationship between sCD14 and ECC is recently revealed by Biria *et al*.[32]Therefore, sCD14 can be viewed as a potentially promising biomarker of ECC. The level of sCD14 can be used as a molecular cue to differentiate carious from noncarious lesions in clinical setting, and in monitoring or evaluating the effectiveness of cariostatic treatment.

Soluble or salivary CD14 is a bacterial pattern recognition receptor (PRR) known to be secreted into the saliva by the salivary glands in a constitutive manner.[33]Other study suggests that sCD14 act as immunological component against bacterial LPS, by forming CD14-LPS complex. This complex can be generated under LPS-binding protein (LBP)-independent mechanism, with LBP acting as a catalysator of CD14-LPS complex genesis.[34]Soluble CD14 is also known to facilitate internalization of *Actinobacillusactinomycetemcomitans* (a periodontophatic species) by human oral epithelial cells *in vitro*, using acytoskeleton-dependent mechanism and up-regulate IL-8 synthesis.[35] Although these later findings further underline the role of sCD14 as an important player of innate immune response "symphony" in oral region, up to date, the occurrence of similar cellular event directed against *S.mutans* is unknown. Overall, this body of evidences put sCD14 in the limelight as an essential molecule in oral homeostasis, withfurther investigations being awaited to reveal its exact immunological role in caries pathobiology.

CONCLUSION

Some promising innate immunity markers associated with ECC / Early Childhood Caries or S-ECC / Severe-Early Childhood Caries are lysozyme, lactoferrin, IL-8, CD36 of salivaryneutrophil, and sCD14. Lowlevels of these biomarkers are associated with carious phenotype in children (as summarized in table 1, and with the oral sources and genetic aspects being summarized in table 2). Further studies are warranted in revealing the complete picture (complex "spider web" of interactomes) of these moleculesin the pathogenesis of ECC and S-ECC, and how the profiles relate to the clinical outcomes.

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