ความสัมพันธ์ระหว่างโรคปริทันต์อักเสบชนิดเรื้อรังกับเอสเอสทีทูในซีรัมซึ่งเป็นสารบ่งชี้ทางชีวภาพของโรคหัวใจชนิดใหม่ ในประชากรไทย

นางสาวดิษยวดี เกตุทัต

จุฬาลงกรณ์มหาวิทยาลัย
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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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ปีการศึกษา 2558
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ASSOCIATION BETWEEN CHRONIC PERIODONTITIS AND SERUM sST2, A NOVEL CARDIAC BIOMARKER, IN A THAI POPULATION

Miss Dissayawadee Katudat

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Periodontics

Department of Periodontology

Faculty of Dentistry

Chulalongkorn University

Academic Year 2015

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Thesis Title	ASSOCIATION BETWEEN CHRONIC PERIODONTITIS AND SERUM sST2, A NOVEL CARDIAC BIOMARKER, IN A THAI POPULATION
By	Miss Dissayawadee Katudat
Field of Study	Periodontics
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ดิษยวดี เกตุทัต : ความสัมพันธ์ระหว่างโรคปริทันต์อักเสบชนิดเรื้อรังกับเอสเอสทีทูในซีรัมซึ่งเป็นสารบ่งชี้ทางชีวภาพของโรคหัวใจชนิดใหม่ ในประชากรไทย (ASSOCIATION BETWEEN CHRONIC PERIODONTITIS AND SERUM sST2, A NOVEL CARDIAC BIOMARKER, IN A THAI POPULATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ทญ. ดร.รังสินี มหานนท์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ทพ. ดร.กิตติ ต.รุ่งเรือง, 48 หน้า.

โรคปริทันต์อักเสบชนิดเรื้อรังคือโรคที่มีการอักเสบของเนื้อเยื่อรองรับฟัน ซึ่งไซโทไคน์ที่เกี่ยวกับการอักเสบจากรอยโรคปริทันต์สามารถหลุดเข้าสู่กระแสเลือดและทำให้เกิดการอักเสบทั่วร่างกายได้ สำหรับเอสเอสทีทูเป็นสารบ่งชี้ทางชีวภาพของโรคหัวใจถูกหลั่งเพื่อตอบสนองต่อการอักเสบ ดังนั้นวัตถุประสงค์ของการศึกษานี้เพื่อศึกษาว่าโรคปริทันต์อักเสบชนิดเรื้อรังมีความสัมพันธ์กับการเพิ่มระดับเอสเอสทีทูในซีรัมของประชากรไทยหรือไม่ โดยมีผู้เข้าร่วมการศึกษา 1,872 คน อายุเฉลี่ย 59.4±4.6 ปี ซึ่ง 209 คนได้รับการวินิจฉัยว่าไม่เป็นหรือเป็นโรคปริทันต์อักเสบระดับน้อย ในขณะที่อีก 984 และ 679 คน ได้รับการวินิจฉัยว่าเป็นโรคปริทันต์อักเสบระดับปานกลางและระดับรุนแรงตามลำดับ ค่ามัธยฐานของเอสเอสทีทูมีค่า 18.1 นาโนกรัม/มิลลิลิตร โดยในเพศชายมีค่าสูงกว่าเพศหญิง (18.8 เทียบกับ 15.7 นาโนกรัม/มิลลิลิตร) การวิเคราะห์การถดถอยเชิงเส้นแสดงให้เห็นว่าระดับเอสเอสทีทูมีความสัมพันธ์กับเพศ แกมมากลูตามิลย์ทรานสเฟอเรส แอสปาร์เทต อะมีโนทรานสเฟอเรส แอลบูมิน เบาหวานและไตรกลีเซอไรด์ โดยเอสเอสทีทูในเพศชายมีความสัมพันธ์กับแกมมากลูตามิลย์ทรานสเฟอเรส แอสปาร์เทต อะมีโนทรานสเฟอเรส แอลบูมิน เบาหวานและไตรกลีเซอไรด์ เอสเอสทีทูในเพศหญิงมีความสัมพันธ์กับแอสปาร์เทต อะมีโนทรานสเฟอเรส แอลบูมิน เบาหวาน ไขมันชนิดความหนาแน่นสูงและโรคปริทันต์อักเสบชนิดเรื้อรังระดับปานกลางและระดับรุนแรง ผลการศึกษานี้แสดงให้เห็นว่าเอสเอสทีทูมีความสัมพันธ์กับโรคปริทันต์อักเสบชนิดเรื้อรังเฉพาะในเพศหญิง

ภาควิชา ปริทันตวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา ปริทันตศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5775807632 : MAJOR PERIODONTICS

KEYWORDS: PERIODONTITIS / CARDIOVASCULAR DISEASES / SERUM SST2

DISSAYAWADEE KATUDAT: ASSOCIATION BETWEEN CHRONIC PERIODONTITIS AND SERUM sST2, A NOVEL CARDIAC BIOMARKER, IN A THAI POPULATION. ADVISOR: PROF. RANGSINI MAHANONDA, Ph.D., CO-ADVISOR: ASSOC. PROF. KITTI TORRUNGRUANG, Ph.D., 48 pp.

Chronic periodontitis is an inflammatory disease of tooth supporting tissues. Inflammatory cytokines produced from periodontitis lesions could leak into circulation and cause systemic inflammation. Soluble ST2 (sST2) is a cardiac biomarker that is secreted in response to systemic inflammation. Therefore, this study aimed to examine whether chronic periodontitis is associated with an increased level of serum sST2 in a Thai population. The study subjects comprised 1,872 individuals with mean age of 59.4±4.6 years. Of these, 209 individuals were diagnosed with no/mild chronic periodontitis, while 984 and 679 individuals were diagnosed with moderate and severe chronic periodontitis, respectively. The median sST2 concentration was 18.1 ng/ml. The concentration was higher in male than in female (18.8 versus 15.7 ng/ml). Linear regression analysis demonstrated that sST2 levels were associated with sex, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), albumin, diabetes mellitus and triglycerides. The concentration of sST2 in male was associated with GGT, AST, albumin, diabetes mellitus and triglycerides. The sST2 in female subjects was associated with AST, albumin, diabetes mellitus, high density lipoprotein, moderate chronic periodontitis and severe chronic periodontitis. The findings of the present study indicated that sST2 was associated with chronic periodontitis only in female subjects.

Department:	Periodontology	Student's Signature .....
Field of Study:	Periodontics	Advisor's Signature .....
Academic Year:	2015	Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude and appreciation to my adviser, Professor Dr. Rangsini Mahanonda, and my co-adviser, Associate Professor Kitti Torrungruang, for guidance, encouragement, supervision, suggestion, correction of this thesis and kindness throughout the course of the Master degree program. I also would like to express my sincere appreciation to Professor Piyamitr Sritara, Dean of the Faculty of Medicine at Ramathibodi Hospital, Mahidol University, for providing the information, valuable guidance and being thesis committee member. I wish to thank Dr. Supreda Suphanantachat for her suggestion and kindness in being chairman of committee member.

My sincere appreciation is also extended to residents of the graduate Periodontics Program and faculty and staff at Ramathibodi Hospital for their assistance in examinations and data entry.

This research was financially supported by a grant from Chulalongkorn Academic Advancement into Its 2nd Century Project.

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LIST OF ABBREVIATIONS

A. actinomycetemcomitans	*Aggregatibacter actinomycetemcomitans*
ACVDs	Atherosclerotic cardiovascular diseases
Apo E	Apolipoprotein E
AST	Aspartate aminotransferase
BMI	Body mass index
CAL	Clinical attachment level
CDC/AAP	Centers for Disease Control and Prevention/American Academy of Periodontology
CRP	C-reactive protein
CVDs	Cardiovascular diseases
DNA	Deoxyribonucleic acid
EFP/AAP	European Federation of Periodontology/American Academy of Periodontology
EGAT	Electricity Generating Authority of Thailand
ELISA	Enzyme-linked immunosorbent assay
GGT	Gamma-glutamyl transferase
HDL	High density lipoprotein
IFN	Interferon
IL	Interleukin
IL-1R	Interleukin-1 receptor
IL-1RAcP	Interleukin-1 receptor accessory protein
IQR	Interquartile ranges
LDL	Low density lipoprotein
LPS	Lipopolysaccharide

MAPK	Mitogen-activated protein kinase
mg/L	Milligram per liter
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng/mL	Nanogram per milliliter
oxLDL	Oxidized low density lipoprotein
PBMC	Peripheral blood mononuclear cell
PD	Probing depth
P. gingivalis	*Porphyromonas gingivalis*
PLD	Phospholipase D
RANKL	Receptor activator of nuclear factor kappa B ligand
SD	Standard deviation
SMCs	Smooth muscle cells
SpHK	Sphingosine kinase
sST2	Soluble suppression of tumerogenicity 2
ST2	Suppression of tumerogenicity 2
ST2L	Transmembrane suppression of tumerogenicity 2
T. forsythia	*Tannerella forsythia*
T_H1	T helper 1
T_H2	T helper 2
TIR	Toll/Interleukin-1-receptor
TLRs	Toll-like receptors
TNF	Tumor necrosis factor

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CHAPTER I

INTRODUCTION

1. Background of the present study

Chronic periodontitis is an inflammatory disease caused by subgingival plaque microorganisms and progresses due to host immuno-inflammatory response, resulting in periodontal destruction and chronic inflammation. Epidemiological studies have shown that periodontitis patients had increased risk of atherosclerotic cardiovascular diseases (ACVDs) especially in males and subjects younger than 65 years old (Dietrich et al., 2013). Evidence suggests that pro-inflammatory cytokines locally produced from periodontitis lesions such as IL-1α, IL-1β and TNF-α could leak into blood stream, resulting in systemic inflammation (Li et al., 2000), and thus could have an indirect effect on atherosclerosis (Libby, 2006). Several studies had been trying to link both diseases by observing inflammatory biomarkers (D'Aiuto et al., 2004, Paraskevas et al., 2008, Slade et al., 2000). One of the most studied biomarkers is C-reactive protein (CRP). The serum levels of CRP were increased in subjects with periodontitis when compared to healthy subjects (D'Aiuto et al., 2004). The elevated CRP levels in periodontitis patients are generally above the level shown in epidemiological and intervention studies to be associated with the risk of cardiovascular diseases (CVDs) (Ridker and Silvertown, 2008).

ST2 is a member of the toll-like/interleukin-1 receptor (IL-1R) superfamily. It has two main isoforms; transmembrane ST2 (ST2L) and soluble ST2 (sST2) (Ciccone et al., 2013). ST2L binding with IL-33 results in cardioprotection by reducing fibrosis, cardiac hypertrophy and myocyte apoptosis (Sanada et al., 2007). However, sST2 acts as a decoy receptor, preventing those beneficial effects of IL-33 to the heart. As a result, the

serum levels of sST2 have been used as a negative prognosis biomarker of CVDs (Demyanets et al., 2014, Mueller et al., 2008, Pascual-Figal et al., 2009). A study in a Thai population showed that sST2 was a good prognostic marker to predict mortality of heart disease and its ability was superior to CRP (Chanyavanich et al., 2014). The sST2 is highly expressed in lung alveolar epithelial cells and cardiac myocytes (Mildner et al., 2010). An *in vitro* study showed that cardiac myocytes and alveolar epithelial cells incubated with IL-1α, IL-1β, TNF-α and supernatants derived from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cell (PBMC) had an increased sST2 secretion (Mildner et al., 2010). Moreover, in healthy subjects who were administrated with LPS, inflammatory cytokines, including IL-6 and TNF-α were elevated in 4 hours, followed by a massive augmentation of sST2 secretion in 24 hours. Because IL-1, IL-6 and TNF-α are commonly found in subjects with chronic periodontitis, it is reasonable to hypothesize that serum sST2 levels may be elevated in subjects with chronic periodontitis in response to these cytokines. Nevertheless, linking of periodontitis and serum sST2 levels in community-based studies has not been done before. Therefore, the objective of this research is to examine the association between periodontal disease severity and serum sST2 levels in a Thai population.

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2. Objectives

To evaluate the association between periodontal status and levels of serum sST2 in a Thai population.

3. Hypothesis

The serum sST2 levels are elevated in subjects with moderate or severe chronic periodontitis compare to those with no/mild disease.

4. Field of research

Epidemiological study/Cross-sectional study

5. Inclusion criteria

Employees of the Electricity Generating Authority of Thailand (EGAT) who worked at the EGAT headquarters in Nontaburi were included in this study. Subjects who had to take antibiotic prophylaxis before periodontal examinations were excluded (Torrungruang et al., 2005). These individuals were subjects who were at risk for bacterial endocarditis, and for hematogenous total joint infection and those undergoing hemodialysis.

6. Application and expectation of research

This study may provide a link for the association between chronic periodontitis and CVDs.

7. Keywords

Periodontitis, cardiovascular diseases, serum sST2

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CHAPTER II

LITERATURE REVIEW

1. Chronic periodontitis

Chronic periodontitis is an inflammatory disease caused by subgingival plaque microorganisms and characterized by progressive destruction of tooth supporting tissues. Characteristics of chronic periodontitis include gingival recession, pocket formation, tooth mobility and tooth loss. A recent systematic review of studies from 37 countries has shown that the 11.2% of individuals aged 15 years or older have severe periodontitis, and the prevalence increases with age (Kassebaum et al., 2014). Chronic periodontitis begins with deposition of bacterial plaque on tooth surface and progresses due to host immunoinflammatory response to plaque bacteria (Figure 1) (Page and Kornman, 1997). Moreover, both genetic and environmental/acquired risk factors such as smoking play an important role in an individual's response to infection of periodontium. The bacterial pathogens most strongly involved in chronic periodontitis are *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia (T. forsythia)*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)* (Socransky et al., 1998, Page and Kornman, 1997). However, there is accumulating evidence indicating that several bacteria, other than these well-known bacteria, are associated with periodontitis such as *Parvimonas micra (*formerly known as *Peptostreptococcus micros), Filifactor* and *Selemonas* (Kumar et al., 2005). The interaction between host immunity and periodontal pathogens results in releasing of many cytokines and enzymes by host cells (Page and Kornman, 1997). The cytokines and enzymes such as IL-1, IL-6, TNF-α and matrix metalloproteinase lead to periodontal destruction and chronic inflammation.

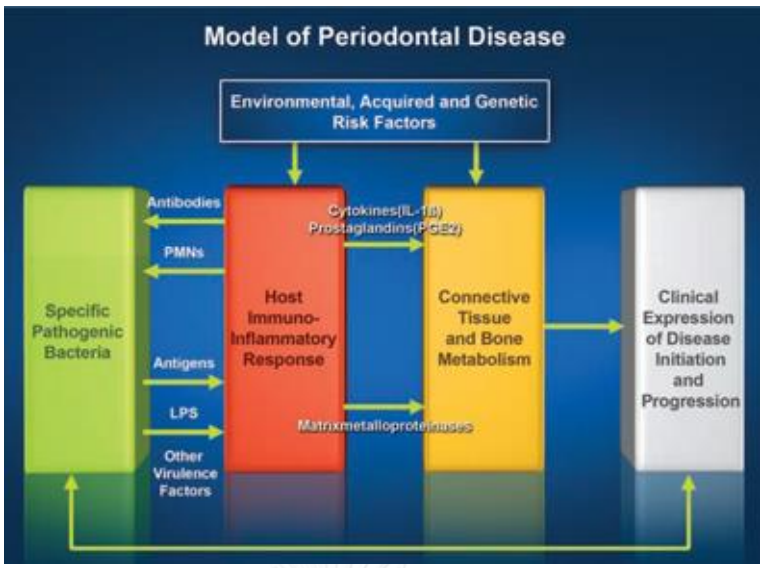

Figure 1: The pathogenesis of chronic periodontitis. Periodontal pathogens activate host immunoinflammatory response, which leads to connective tissue and bone destruction. Genetic and environmental/acquired risk factors act as modifiers that influence the nature and progression of disease in each individual (Page and Kornman, 1997).

Although periodontitis is a disease of tooth-supporting tissues, epidemiological evidence suggests that it may also be associated with an increased risk of systemic diseases and adverse medical outcomes such as ACVDs (Dietrich et al., 2013), diabetes mellitus (Chapple and Genco, 2013) and preterm low-birth weight (Sanz and Kornman, 2013). However, the biological mechanisms linking between periodontitis and organs distant from oral cavity are still unclear. Two mechanisms have been proposed:

1. **Direct bacterial action.** The surface area of ulcerated epithelium lining periodontal pockets in patients with generalized periodontitis has been estimated to range from 8 to 20 cm^2 (Hujoel et al., 2001). This ulceration provides a portal for subgingival plaque bacteria or endotoxin to enter into blood circulation, causing bacteremia or endotoxinemia. Blood levels of endotoxin were increased during mastication, and were significantly higher in subjects with severe periodontitis than in

those with mild or moderate disease (Garcia et al., 2001, Geerts et al., 2002). In addition, the risk of bacteremia due to periodontal probing was higher in untreated periodontitis subjects than in subjects with gingivitis (Daly et al., 2001). Moreover, a systematic review reported that the amount of plaque accumulation and gingival inflammation was associated with the prevalence of bacteremia (Tomas et al., 2012). A more recent systematic review also indicated that bacteremia was observed in 49.4 percent of patients after periodontal procedures, including periodontal probing, non-surgical periodontal therapy and periodontal surgery (Horliana et al., 2014). The bacteria frequently found in the blood circulation were *Streptococcus viridans, A. actinomycetemcomitans, P. gingivalis*, *Parvimonas micra*, *Streptococcus spp.,* and *Actinomyces spp.*

2. Indirect bacterial action: Systemic inflammation. Another possible link between periodontitis and systemic diseases is inflammation. In patients with periodontitis, cells within connective tissues underlying periodontal pockets secrete several inflammatory cytokines such as IL-6, TNF-α, and IL-1β, which could enter into the blood circulation and affect distant organs (Li et al., 2000). Serum levels of IL-6 and TNF-α have been shown to be significantly higher in subjects with periodontitis than healthy controls (Gumus et al., 2014, Loos et al., 2000). In addition, there is an association between periodontitis and CRP, a systemic inflammatory marker produced by liver in response to the elevation of IL-6 levels. A meta-analysis of 10 cross-sectional studies showed that serum CRP levels in subjects with chronic periodontitis were 1.56 mg/L higher than those of periodontally healthy subjects (Paraskevas et al., 2008, Slade et al., 2000). Evidence from 6 treatment studies also showed that periodontal therapy lowered the levels of serum CRP by 0.50 mg/L (95% CI 0.08-0.93).

2. ACVDs

CVDs, diseases of the heart and/or blood vessels, are an important cause of death and the mortality rate of CVDs increases every year worldwide. In Thailand, the mortality rate per 100,000 populations was increased from 61.94 in 2011 to 84.38 in 2013 (กระทรวงสาธารณสุข, 2558). CVDs include numerous problems, the majority of which are related to the process called atherosclerosis. This group of CVDs, known as ACVDs, consists of fatal and non-fatal coronary heart disease (angina, myocardial infarction), ischemic cerebrovascular disease (stroke/transient ischemic attack), and peripheral arterial disease.

Atherosclerosis is a chronic immuno-inflammatory disease of large and medium-sized arteries that is characterized by narrowing of the vessels (Falk, 2006). There are three stages in the development of atherosclerotic plaque: initiation, progression and end stage thrombotic complication (Libby et al., 2011). The disease initiation involves leukocyte adhesion to the activated endothelial cells, migration of leukocytes, especially monocytes, into the intima, maturation of monocytes into macrophages, and their uptake of oxidized low density lipoprotein (oxLDL), yielding foam cells. Lesion progression steps include the migration of smooth muscle cells (SMCs) from tunica media to the intima, their proliferation and increased production of extracellular matrix, leading to thickening of vessel walls. Extracellular lipid derived from dead and dying macrophages and SMCs accumulates in the central region of a plaque, forming the lipid or necrotic core. Finally, a physical disruption of the plaque's fibrous cap enables blood coagulation factors to come into contact with the plaque's interior, leading to thrombus formation in the vessel lumen, where it can impede blood flow.

Inflammation involves in all three stages of atherosclerosis. The early stage is triggered by inflammatory processes in response to arterial injury (Libby, 2006). The injurious factors to blood vessels include high saturated-fat diet, smoking, hypertension,

hyperglycemia and infection (Rosenfeld and Campbell, 2011, Libby, 2006). An infection has been proposed for many years that it may play a role in the pathogenesis of atherosclerosis (Rosenfeld and Campbell, 2011). The pathogens including *Chlamydia pneumoniae*, *P. gingivalis*, *Helicobacter pylori*, influenza A virus and hepatitis C virus have been shown to induce inflammation at vessels. The release of local pro-inflammatory cytokines such as IL-1, TNF-α increases vascular permeability and attracts blood monocytes to the vessel wall (Libby, 2006). Monocytes recruited to the intima express scavenger receptors that permit the uptake of oxLDL and transform into foam cells. These cells can produce pro-inflammatory mediators, reactive oxygen species, and tissue factor pro-coagulants that amplify local inflammation and promote thrombus formation. Although fewer in number than monocytes, T cells also enter the intima and play an important role in atherosclerosis. After antigen-specific activation, T helper 1 (T_H1) cells secrete cytokines and growth factors, which can activate macrophages and increase migration and proliferation of SMCs from tunica media (Hansson, 2001). Finally, the inflammation can promote thrombus formation by interfering with the strength of fibrous cap. T lymphocytes and macrophages in the plaque lesions release cytokines and enzymes, which result in increased collagen degradation and reduced new collagen formation of the vessel wall (Libby, 2006).

3. Biomarkers in CVDs

The traditional risk factors including smoking, diabetes mellitus, hyperlipidemia and hypertension have been involved in the pathogenesis of CVDs and have been used to predict the risk of CVDs (Khot et al., 2003). However, it has been shown that 15% of female patients and 19% of male patients with coronary heart disease lacked all four of these traditional risk factors. In addition, 37% of female and 43% of male patients had only one of these traditional risk factors. Therefore, using only traditional risk factors may

lead to underdiagnosis and inaccurate disease prediction. To date, cardiac biomarkers have been introduced as a tool for risk stratification, treatment selection and monitoring, and disease prevention for CVDs. Cardiac biomarkers can be divided into 6 groups (Braunwald, 2008, Iqbal et al., 2012) as follows:

3.1 Inflammation group: The inflammation process is important in pathogenesis and progression of CVDs. The biomarkers in this group include CRP, TNF-α and IL-6. A population-based prospective study has shown that after adjustment for conventional CVDs risk factors, serum levels of CRP and TNF-α were significant, independent predictors of coronary heart disease, all CVDs events and total mortality among men (Tuomisto et al., 2006).

3.2 Oxidative stress group: The imbalance between reactive oxygen species and antioxidants contribute to the pathogenesis of cardiac and endothelial dysfunction (Ungvari et al., 2005). Biomarkers in this group include plasma myeloperoxidase, urinary biopyrrins and oxLDL.

3.3 Extracellular-matrix remodeling group: The imbalance of ventricular remodeling can impair ventricular function (Braunwald, 2008). Biomarkers in this group include matrix metalloproteinase, propeptide procollagen type I and plasma procollagen type III.

3.4 Neurohormones group: Activation of sympathetic nervous system and neurohormonal disturbance might lead to heart failure (Braunwald, 2008). Biomarkers in this group include norepinephrine, endothelin, renin, and angiotensin II.

3.5 Myocyte injury group: Severe cardiac ischemia or stress on myocytes caused by inflammation, oxidative stress or neurohormonal activity may lead to myocyte injury (Braunwald, 2008). Biomarkers in this group include high sensitivity troponin, myosin light-chain kinase I and creatine kinase MB fraction.

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3.6 Myocyte stress group: Biomarkers in this group are secreted in response to increased mechanical load and cardiac wall stretch. Biomarkers in this group include brain natriuretic peptide, N-terminal pro-brain natriuretic peptide and sST2. However, serum sST2 levels were not only increased by myocyte stress but also by inflammation (Mildner et al., 2010, Pascual-Figal and Januzzi, 2015).

4. Association between periodontitis and ACVDs

Associations between periodontitis and ACVDs have been studied for many years. Recently, the consensus report of the joint European Federation of Periodontology/American Academy of Periodontology (EFP/AAP) workshop in 2013 concluded that there is consistent and strong epidemiologic evidence supporting the association between periodontitis and increased risk for future ACVDs (Tonetti and Van Dyke, 2013, Dietrich et al., 2013). This association was independent of established cardiovascular risk factors (Dietrich et al., 2013). The risk was stronger in subjects younger than 65 years old, and was greater for stroke than for coronary heart disease. However, there was limited evidence to support the association between periodontitis and secondary cardiovascular events. With regard to the effects of periodontal treatment on ACVDs, there is moderate evidence indicating that periodontal therapy improves both clinical and surrogate outcomes of endothelial function (D'Aiuto et al., 2013). However, there are no studies up-to-date for the effects of periodontal therapy on primary ACVDs prevention.

In vitro, animal and clinical studies indicate that there are two possible biological mechanisms that link periodontitis to ACVDs (Kholy et al., 2015):

1. Direct bacterial action: It has been hypothesized that bacterial pathogens from periodontitis lesions can enter blood stream and initiate pathologic changes of blood vessel walls, leading to atherosclerosis (Reyes et al., 2013). A recent

study also proposes that phagocytic cells could deliver periodontal bacteria from diseased pockets to the affected tissue (Carrion et al., 2012). Using immunohistochemistry and polymerase chain reaction, several periodontal pathogens have been found in human atherosclerotic lesions (Mahendra et al., 2013, Haraszthy et al., 2000, Figuero et al., 2011). These include *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. In addition, there was a correlation between the pathogens found in subgingival plaque and those detected in the vascular lesions (Mahendra et al., 2013). However, there is limited evidence supporting that live periodontal pathogens (*P. gingivalis* and *A. actinomycetemcomitans*) could be detected in human atheromatous tissues (Kozarov et al., 2005). Furthermore, several animal models provide supportive evidence for this hypothesis. Mice infected with *P. gingivalis* had an increased size and faster progression of atherosclerotic lesions than non-infected mice (Li et al., 2002, Lalla et al., 2003). *P. gingivalis* DNA was also found within aorta and other tissues in these mice. In addition, immunization with heat-killed *P. gingivalis* or its antigen prior to infection in mice could prevent the accelerated progression of atherosclerotic lesions (Li et al., 2002).

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2. Indirect bacterial action: There is evidence that periodontal infection leads to increases in systemic inflammatory mediators and thus could have an indirect effect on atherosclerosis (Schenkein and Loos, 2013). One of the important markers of systemic inflammation is CRP. It has been shown that CRP levels were increased by two folds in patients who had either periodontitis or CVDs, and by three folds in those who had both diseases (Glurich et al., 2002). The elevated CRP levels observed in periodontitis patients are generally above the level shown in epidemiological and intervention studies to be associated with the risk of CVDs (Ridker and Silvertown, 2008). Moreover, subjects with severe periodontitis experienced a greater risk of having

CRP-associated CVDs than those with mild disease (D'Aiuto et al., 2004, Paraskevas et al., 2008). Periodontal treatment reduced serum CRP levels and decreased the number of subjects with a medium and high CRP-associated CVDs risk. From a recent systematic review, there is moderate evidence supporting that periodontal therapy reduces systemic inflammation as evidenced by reduction in serum IL-6 and CRP (D'Aiuto et al., 2013). Therefore, systemic inflammation may provide a link between periodontitis and ACVDs.

5. ST2

5.1 ST2 isoforms

ST2 or suppression of tumerogenicity 2 (also known as T1, DER4, Fit-1, or IL1RL1) is a member of the Toll/Interleukin-1-receptor (TIR) superfamily (Ciccone et al., 2013). The *ST2* gene is located on chromosome 2q12 and is part of the larger *IL-1* gene cluster. ST2 has two main isoforms: transmembrane (ST2L) and soluble (sST2) forms. The ST2L is a membrane-bound isoform comprising an extracellular domain of three linked immunoglobulin-like motifs, a transmembrane domain, and an intracellular TIR domain that is homologous to toll-like receptors (TLRs) and other members of IL-1 receptor family (Figure 2). The sST2 lacks the transmembrane and intracellular domains and is secreted into bloodstream. ST2L is expressed by many cell types. The expression is highest on T helper 2 (T_H2) cells and mast cells (Xu et al., 1998, Kakkar and Lee, 2008). In contrast, sST2 is highly expressed in lung alveolar epithelial cells and cardiac myocytes (Mildner et al., 2010).

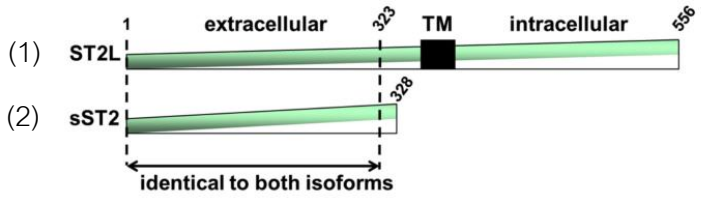

Figure 2: The *ST2* gene encodes 2 main protein isoforms: (1) ST2L is a transmembrane receptor, consisting of an extracellular domain of three linked immunoglobulin-like motifs, a transmembrane segment (TM) and an intracellular cytoplasmic domain, and (2) sST2 is a soluble isoform which lacks the transmembrane and cytoplasmic domains (Pascual-Figal and Januzzi, 2015).

5.2 IL-33 and ST2 interaction

Interleukin-33 (IL-33), a member of the IL-1 family cytokines, is a functional ligand of ST2L. IL-33 is expressed by multiple organs and cell types, mainly in fibroblasts, SMCs, epithelial cells, and endothelial cells (Schmitz et al., 2005). Binding between IL-33 and a receptor complex consisting of ST2L and interleukin-1 receptor accessory protein (IL-1RAcP) can trigger at least two signaling pathways: the mitogen-activated protein kinase (MAPK) pathway and the phospholipase D (pLD)–sphingosine kinase (SpHK) pathway that leads to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Figure 3) (Liew et al., 2010). The outcome of these events is the production of pro-inflammatory cytokines and chemokines such as IL-1β, IL-5, IL-13 and TNF. sST2 binds to IL-33 and acts as a 'decoy' receptor for IL-33, thereby inhibiting the interaction between IL-33 and ST2L.

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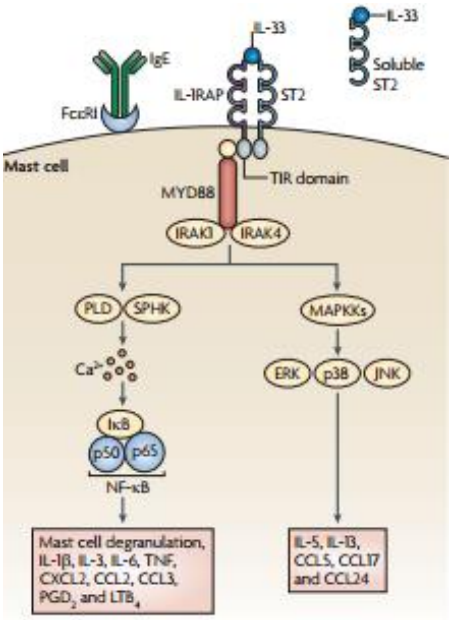

Figure 3: IL-33/ST2 Signaling. IL-33 signaling through ST2L and interleukin-1 receptor accessory protein (IL-1RAcP) leads to activation of at least two independent pathways: the phospholipase D (PLD)-sphingosine kinase (SPHK) pathway and the mitogen-activated protein kinase (MAPK) pathway. These two pathways may act synergistically to induce gene expression leading to cytokine and chemokine synthesis. The sST2 binds to IL-33 and acts as decoy receptor for IL-33 (Liew et al., 2010).

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5.3 IL-33/ST2 signaling in inflammatory diseases

The expression of IL-33 and ST2 is upregulated in response to inflammation, infection or immunological challenge (Schmitz et al., 2005, Kumar et al., 1997). The role of IL-33/ST2 in inflammatory diseases is T_H2 dependent (Pascual-Figal and Januzzi, 2015). Binding of IL-33 to ST2L activates T_H2 effector cells and the production of T_H2 cytokines including IL-4, IL-5 and IL-13 (Schmitz et al., 2005). On the other hand, sST2 acts as a decoy receptor, preventing IL-33 from binding to ST2L (Hayakawa et al., 2007). Therefore, sST2 is involved in the attenuation of T_H2

inflammatory response. Evidence show that IL33/ST2L may play a role in the pathogenesis of several diseases associated with T_H2 response such as asthma (Oshikawa et al., 2001), rheumatoid arthritis (Xu et al., 2013) and inflammatory bowel diseases (Nunes and Bernardazzi, 2014).

5.4 IL-33/ST2 signaling in CVDs

Disease conditions that increase myocardial stress, such as myocardial infarction, hypertension and valvular disease, could lead to myocyte hypertrophy and ventricular fibrosis. Experimental models have shown that IL-33/ST2L signaling plays a cardioprotective role by reducing myocardial fibrosis, preventing cardiomyocyte hypertrophy, and improving myocardial function (Pascual-Figal and Januzzi, 2015). Both cardiac fibroblasts and cardiomyocytes express IL-33 and ST2, and the expression is upregulated in response to myocardial stress or injury (Weinberg et al., 2002). In mice subjected to pressure overload, IL-33 treatment reduced myocardial hypertrophy and fibrosis and improved survival (Sanada et al., 2007). Moreover, treatment with IL-33 prevented cultured cardiomyocytes from apoptosis, and reduced infarct size and fibrosis in rat models with myocardial infarction (Seki et al., 2009). In mice lacking ST2, the benefits of IL-33 were lost, resulting in left ventricular hypertrophy, more chamber dilation, reduced fractional shortening, more fibrosis and impaired survival (Sanada et al., 2007).

In contrast to the cardioprotective role of IL-33, sST2 functions as a decoy receptor for IL-33, thereby antagonizing the beneficial effect of IL-33/ST2L signaling (Seki et al., 2009, Sanada et al., 2007). The role of sST2 as a negative prognosis biomarker of CVDs is supported by several clinical studies showing that

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serum sST2 levels were increased in patients with acute myocardial infarction and heart failure (Demyanets et al., 2014, Mueller et al., 2008, Pascual-Figal et al., 2009).

In addition to the role in cardiac remodeling, IL-33/ST2L signaling also plays a protective role in atherosclerosis. T_H1 is a predominant cell in atherosclerotic plaque and promotes atherosclerotic formation (Hansson, 2001). However, IL-33/ST2L signaling induces a switch in immune response from T_H1 to T_H2. In Apolipoprotein $E^{-/-}$ (Apo $E^{-/-}$) mice on a high-fat diet, treatment with IL-33 led to a smaller aortic atherosclerotic plaque and increased T_H2 cytokines compared to the control group (Figure 4) (Miller et al., 2008, Kakkar and Lee, 2008). In contrast, the mice treated with sST2 showed increases in atherosclerotic plaque size and T_H1 cytokines. A subsequent study demonstrated that the protective effect of IL-33 was mediated by a decrease in lipid uptake and an increase in lipid efflux of macrophages, resulting in less foam cell accumulation (McLaren et al., 2010).

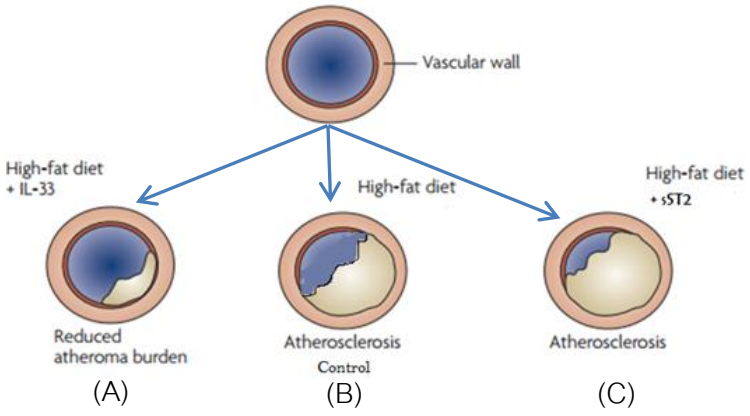

Figure 4: IL-33 and sST2 in atherosclerotic plaque formation: (A) Apo $E^{-/-}$ Mice fed with a high-fat diet and treated with IL-33, **(B)** Apo $E^{-/-}$ Mice fed with a high-fat diet (the control group) and **(C)** Apo $E^{-/-}$ Mice fed with a high-fat diet and treated with sST2 (modified from (Kakkar and Lee, 2008)).

5.5 Role of sST2 cardiac biomarker

Many studies have shown that an increase in sST2 levels is associated with a worse prognosis in patients with existing CVDs (Demyanets et al., 2014, Pascual-Figal et al., 2009, Mueller et al., 2008). Moreover, in populations free of CVDs, sST2 is a strong predictor of cardiovascular outcomes independent of other cardiovascular biomarkers, and its ability is superior to traditional cardiovascular risk factors. In the Framingham Heart Study in which subjects were followed for a mean of 11.3 years, sST2 was associated with an increased risk of all-cause mortality, incident heart failure and major cardiovascular events, including myocardial infarction, coronary insufficiency, heart failure and stroke (Wang et al., 2012). Similarly, a 10-year cohort study of the employees of the EGAT showed that sST2 was a strong predictor of cardiovascular death with a hazard ratio of 4.2 (95% CI: 1.1-16.9) (Chanyavanich et al., 2014). In addition, the ability of sST2 to predict cardiovascular death was superior to that of CRP.

Few studies have examined factors that influence the serum levels of sST2 in the general population. In the Framingham Heart Study, higher sST2 levels were observed in males, older subjects, and those with diabetes mellitus or hypertension (Coglianese et al., 2012). There were differences observed between men and women with regard to serum sST2 levels. In men, the higher sST2 levels were associated with diabetes mellitus and systolic blood pressure whereas in women, they were associated with diabetes mellitus, use of antihypertensive drug, age and body mass index (BMI). Although the sST2 levels have been correlated with several traditional cardiovascular risk factors, results from the Framingham study showed that only 14% of variability in sST2 concentrations could be explained by these factors, whereas up to 40% of the variations were due to genetic factors (Ho et al., 2013). Therefore, sST2 may provide information beyond traditional risk factors for the prediction of cardiovascular outcomes.

5.6 IL-33/ST2 signaling in chronic periodontitis

IL-33/ST2 signaling has been linked to several inflammatory diseases such as rheumatoid arthritis and asthma. However, there is limited evidence with regard to its role in chronic periodontitis. The expression of IL-33 in gingival tissues was increased in subjects with chronic periodontitis compared to healthy controls (Malcolm et al., 2015). In the cultured human gingival epithelial cell and oral keratinocyte cell line, IL-33 expression was increased when stimulated with *P. gingivalis* (Awang, 2014). Similarly, IL-33 expression in gingival tissues was elevated in *P. gingivalis*-infected mice compared to uninfected controls (Malcolm et al., 2015). Administration of systemic IL-33 in *P. gingivalis*-infected mice increased the expression of receptor activator of nuclear factor kappa B ligand (RANKL) in gingival tissues and exacerbated alveolar bone loss. Blocking RANKL using osteoprotegerin prevented bone loss in *P. gingivalis*-infected, IL-33 treated mice, suggesting that IL-33/ST2L signaling may involve in bone loss via RANKL pathway.

5.7 Systemic inflammation and serum sST2

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Systemic inflammation is the clinical expression of the action of complex intrinsic mediators, resulting in inflammation of the whole body (Nystrom, 1998). The causes of systemic inflammation may involve both infectious agents such as sepsis and non-infectious agents such as trauma, burns and myocardial infarction. Linking between sST2 and systemic inflammation has been observed in sepsis. In a case-control study, serum sST2 and IL-10 levels were elevated in patients with sepsis (Brunner et al., 2004). The elevation of serum ST2 was not dependent on the source of infection but was correlated with disease severity and mortality (Hoogerwerf et al., 2010). Furthermore, increasing of serum sST2 was higher in patients with bacteremia than in those with viral

infection (Calo Carducci et al., 2014). In *in vivo* study, cardiac myocytes and alveolar epithelial cells incubated with IL-1α, IL-1β, TNF-α and supernatants derived from LPS-stimulated PBMC led to increased sST2 secretion (Mildner et al., 2010). Moreover, healthy subjects who were administrated with LPS showing an elevation of inflammatory cytokines (IL-6 and TNF-α) in 4 hours and followed by a massive augmentation of sST2 secretion in 24 hours. Therefore, Mildner and colleagues hypothesized that the elevation of circulating cytokines in response to infection led to an increase of sST2 secretion from cardiac myocytes and alveolar epithelial cells (Figure 5).

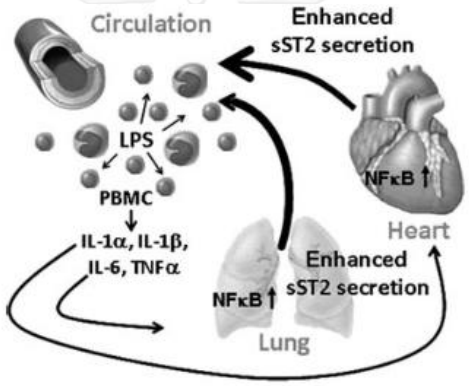

Figure 5: Scheme of the human endotoxin model. Endotoxin stimulation leads to inflammation and increased production of inflammatory cytokines from peripheral blood mononuclear cell (PBMC). Inflammatory cytokines subsequently stimulate cardiac myocytes and alveolar epithelial cells of lung to secrete sST2 (Mildner et al., 2010).

As previously mentioned, chronic periodontitis is an infectious disease that leads to increased systemic inflammatory cytokines such as IL-1α, IL-1β and TNF-α. These cytokines and LPS administration have been shown to promote sST2 secretion (Mildner et al., 2010). Therefore, we hypothesize that serum sST2 levels may be elevated in subjects with chronic periodontitis.

CHAPTER III

MATERIALS AND METHODS

This cross-sectional study was conducted among the EGAT employees in year 2002. The study was approved by the Ethics Review Committee of the Faculty of Medicine at Ramathibodi Hospital, Mahidol University and Faculty of Dentistry at Chulalongkorn University, Bangkok, Thailand. All subjects signed an inform consent prior to the study.

1. Study Design

Medical data were received from Ramathibodi Hospital. Medical examinations consisting of blood and urine analyses, electrocardiogram, chest x-rays and anthropometric measurements. Blood samples were stored at -80^{o}C in year 2002 and were thawed for measurement of sST2 in year 2013. The sST2 levels were measured in citrated plasma using a highly sensitive ELISA (Presage®ST2 assay, Critical Diagnostics, San Diego, CA, USA) which the detection limit was 2 ng/mL (Dieplinger et al., 2009). Sociodemographic characteristics were obtained by a questionnaire.

Periodontal examinations were carried out by four experienced periodontists and three post-graduate students from the Department of Periodontology, Faculty of Dentistry, Chulalongkorn University as described in prior study (Torrungruang et al., 2005). One maxillary quadrant and one contralateral mandibular quadrant were randomly selected. All teeth in these quadrants were examined except third molars and retained roots. The examinations included the number of missing teeth, probing depth (PD) and gingival recession. PD and gingival recession were measured using a PCP-UNC 15 probe on six sites per tooth (mesiobuccal, midbuccal, distobuccal,

mesiolingual, midlingual and distolingual). Clinical attachment level (CAL) was calculated as the sum of PD and gingival recession.

2. Statistical methods

Subjects were categorized into 3 groups according to the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) case definition (Page and Eke, 2007).

1. Group 1 was no/mild chronic periodontitis (< 2 interproximal sites with CAL ≥ 4 mm and <2 interproximal sites with PD ≥ 5 mm)

2. Group 2 was moderate chronic periodontitis (≥ 2 interproximal sites with CAL ≥ 4 mm (not on the same tooth), or ≥ 2 interproximal sites with PD ≥ 5 mm (not on the same tooth))

3. Group 3 was severe chronic periodontitis (≥ 2 interproximal sites with CAL ≥ 6 mm (not on the same tooth) and ≥ 1 interproximal site with PD ≥ 5 mm)

SPSS version 17.0 software (SPSS Inc, Chicago, USA) was used to analyze the data. Continuous variables were tested for normal distribution by Kolmogorov-Smirnov test. Normally distributed data were described by mean and standard deviation (SD). To compare means between three groups, ANOVA and Tukey's post-hoc test were used for analysis. The analysis of difference between two groups was performed using the t-test. Non-normally distributed data were described by median and interquartile ranges (IQR). To compare medians between three groups, Kruskal-Wallis test and Mann-Whitney U test were used for analysis. The analysis of difference between two groups was performed using the Mann-Whitney U test. The associations between categorical variables were analyzed using the chi-square test.

The association of the sST2 with the independent variables was assessed by univariate and multivariate linear regression analysis. For multivariate model, the

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backward method was used. First, all explanatory variables were included in the model. The least significant explanatory variable was then removed one by one from the model until the optimum model was found. The following independent variables were entered into the backward linear regression models: age, sex, education, income, BMI, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), albumin, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, cholesterol, diabetes mellitus, blood pressure, smoking, drinking, plaque score and chronic periodontitis. Statistical differences with P value < 0.05 were considered significant.

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CHAPTER IV

RESULTS

From 2,360 subjects who participated in this study, 2,276 subjects received both medical and dental examinations. We excluded 271 subjects who did not have data on periodontal measurements: 6 subjects who required antibiotic prophylaxis, 54 edentulous subjects, and 211 subjects who had fewer than six teeth present in the selected two quadrants (Torrungruang et al., 2005). We also excluded 133 subjects who did not have data on sST2. Total number of subject included in this study was 1,872. The mean age of subjects was 59.4±4.6 years and 75.6% were male (Table 1). When compared to females, males were older (P = 0.005) and had lower education (P < 0.001) and higher income (P = 0.032). They also had higher blood pressure and higher AST, GGT and triglycerides levels but lower cholesterol, LDL and HDL levels (P < 0.001). Moreover, male had more diabetics, more chronic periodontitis, higher plaque index and were more likely to be smokers and drinkers (P < 0.005).

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Table 1 Characteristics of study subjects[$]

Variables	All subjects (N=1,872)	Male (N=1,416)	Female (N=456)	P value
1. Age	59.4±4.6	59.6±4.8	58.9±4.2	0.005[§]
2. Education level				<0.001[€]
< Bachelor degree	1,067 (57.0%)	859 (60.7%)	208 (45.7%)	
Bachelor degree	670 (35.8%)	456 (32.2%)	214 (46.9%)	
> Bachelor degree	122 (6.5%)	92 (6.5%)	30 (6.5%)	

Table 1 Characteristics of study subjects[$] (continue)

Variables	All subjects (N=1,872)	Male (N=1,416)	Female (N=456)	P value
3. Income (Baht)				0.032[€]
< 10,000	135 (7.2%)	105 (7.4%)	30 (6.6%)	
10,000-19,999	148 (7.9%)	124 (8.8%)	24 (5.3%)	
20,000-49,999	552 (29.5%)	414 (29.2)	138 (30.3%)	
50,000- 99,999	662 (35.4%)	497 (35.1%)	165 (36.2%)	
≥ 100,000	214 (11.4%)	148 (10.5%)	66 (14.5%)	
4. BMI (kg/m^2)				0.117[€]
< 18.5 (underweight)	46 (2.5%)	34 (2.4%)	12 (2.6%)	
18.5- 24.9 (normal)	1,025 (54.8%)	764 (54.0%)	261 (57.2%)	
25-29.9 (overweight)	660 (35.3%)	519 (36.7%)	141 (30.9%)	
≥ 30.0 (obese)	121 (6.5%)	85 (6.0%)	36 (7.9%)	
5. Blood pressure				
SBP	128.0 (115.0-140.0)	130.0 (117.0-141.0)	121.0 (110.0-134.0)	<0.001[¶]
DBP	83.0 (75.7-90.0)	84.0 (78.0-91.0)	80.0 (71.0-87.0)	<0.001[¶]
6.Lipid profiles (mg/dl)				
Cholesterol	240.4±42.6	236.7±41.8	252.2±42.8	<0.001[§]
LDL	156.6±39.0	154.6±38.6	162.7±39.6	<0.001[§]
HDL	52.0 (44.0-62.0)	50.0 (43.0-58.2)	61.0 (51.0-73.7)	<0.001[¶]
Triglycerides	127.0 (93.0-179.0)	131.0 (96.0-187.0)	112.5 (82.0-156.0)	<0.001[¶]
7. AST	23.0 (19.0-29.0)	24.0 (19.0-30.0)	22.0 (18.0-26.0)	<0.001[¶]
8. GGT	26.0 (17.0-45.0)	29.0 (19.0-51.0)	18.0 (13.0-29.0)	<0.001[¶]
9. Albumin	46.7±3.0	46.8±3.02	46.5±3.1	0.115[§]
10. Diabetes mellitus				0.003[€]
Yes	299 (16.0%)	247 (17.4%)	52 (11.4%)	
No	1,565 (83.6%)	1,165 (82.3%)	400 (87.7%)	
11. Periodontitis				<0.001[€]
No/mild	209 (11.2%)	106 (7.5%)	103 (22.6%)	
Moderate	984 (52.5%)	719 (50.8%)	265 (58.1%)	
Severe	679 (36.3%)	591 (41.7%)	88 (19.3%)	

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Table 1 Characteristics of study subjects[$] (continue)

Variables	All subjects (N=1,872)	Male (N=1,416)	Female (N=456)	P value
12. Smoking				<0.001[€]
Non-smoker	888 (47.4%)	453 (32.0%)	435 (95.4%)	
Former smoker	699 (37.3%)	688 (48.6%)	11 (2.4%)	
Current smoker	270 (14.4%)	262 (18.5%)	8 (1.8%)	
13. Alcohol				<0.001[€]
None/occasional drinker	1,027 (54.9%)	607 (42.9%)	361 (92.1%)	
Former drinker	319 (17.0%)	305 (21.5%)	14 (3.1%)	
Current drinker	513 (27.4%)	495 (35.0%)	18 (3.9%)	
14. Plaque index	62.5 (44.4-79.1)	65.3 (46.4-80.7)	54.3 (35.8-73.0)	<0.001[¶]

Abbreviations: BMI = body mass index, SBP= systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein, LDL = low density lipoprotein, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase. [$] Differences in the number of subjects are due to missing data. [€] The association between sex and other categorical variables, analyzed using Chi-square test. [¶] The comparison between male and female, analyzed using Mann-Whitney U test. [§] The comparison between male and female, analyzed using t-test.

Based on CDC/AAP definition (Page and Eke, 2007), 11.2% of subjects were diagnosed with no/mild chronic periodontitis, 52.5% with moderate chronic periodontitis and 36.3% with severe chronic periodontitis. Compared to subjects with no/mild chronic periodontitis, those with severe chronic periodontitis had higher proportion of male, older, less educated and had lower income ($P < 0.001$) (Table 2). In addition, they had a higher proportion of diabetics, smokers, drinkers ($P \leq 0.001$) (Table 3). They also had higher blood pressure ($P < 0.001$), BMI ($P = 0.049$) and plaque index ($P < 0.001$). Moreover, subjects with severe chronic periodontitis also had higher GGT ($P < 0.001$) and triglycerides levels ($P < 0.001$), but lower cholesterol ($P = 0.008$), LDL ($P = 0.019$) and HDL levels ($P < 0.001$).

Table 2 Sociodemographic characteristics of periodontitis subjects[$]

Variables	Periodontitis			P value
	No/mild	Moderate	Severe	
1. Age	58.1±4.3	59.4±4.6	59.7±4.8	<0.001[§]
2. Sex				<0.001[€]
Male	106 (50.7%)	719 (73.1%)	591 (87.0%)	
Female	103 (49.3%)	265 (26.9%)	88 (13.0%)	
3. Education level				<0.001[€]
< Bachelor degree	88 (42.1%)	526 (53.5%)	453 (66.7%)	
Bachelor degree	102 (48.8%)	373 (37.9%)	195 (28.7%)	
> Bachelor degree	19 (9.1%)	77 (7.8%)	26 (3.8%)	
4. Income (Baht)				<0.001[€]
< 10,000	8 (3.8%)	68 (6.9%)	59 (8.7%)	
10,000-19,999	10 (4.8%)	73 (7.4%)	65 (9.6%)	
20,000-49,999	58 (27.8%)	266 (27.0%)	228 (33.6%)	
50,000- 99,999	79 (37.8%)	368 (37.4%)	215 (31.7%)	
≥ 100,000	38 (18.2%)	125 (12.7%)	51 (7.5%)	

[$] Differences in the number of subjects are due to missing data. [€] The association between periodontitis groups and other categorical variables, analyzed using Chi-square test. [§] The comparison between periodontitis groups, analyzed using ANOVA test. * Significant differences between groups at $P < 0.05$, analyzed using Tukey's post-hoc test.

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Table 3 Clinical characteristics of periodontitis subjects[$]

Variables	Periodontitis			P value
	No/mild	Moderate	Severe	
1. BMI (kg/m^2)				0.049[€]
< 18.5 (underweight)	3 (1.4%)	25 (2.5%)	18 (2.7%)	
18.5- 24.9 (normal)	137 (65.6%)	525 (53.4%)	363 (53.5%)	
25-29.9 (overweight)	57 (27.3%)	365 (37.1%)	238 (35.1%)	
≥ 30.0 (obese)	11 (5.3%)	59 (6.0%)	51 (7.5%)	
2. Blood pressure				
SBP	122.0 (110.0-135.0)	128.0(115.0-140.0)	130.0(117.0-142.0)	<0.001[¶]
DBP	80.0 (72.0-89.0)	83.0(75.2-90.0)	84.0 (77.0-91.0)	<0.001[¶]
3. Lipid profiles (mg/dl)				
Cholesterol	246.4±41.3	241.6±42.9	236.9±42.2	0.008[§]
LDL	160.1±37.7	158.2±39.5	153.3±38.4	0.019[§]
HDL	57.0 (46.0-69.0)	53.0 (45.0-63.0)	51.0 (42.0-60.0)	<0.001[¶]
Triglycerides	119.0 (89.0-167.0)	125.0 (91.0-173.7)	133.0 (98.0-194.0)	<0.001[¶]
4. AST	22.0 (19.0-28.0)	23.0 (18.0-29.0)	23.0v(19.0-30.0)	0.463[¶]
5. GGT	23.0 (14.0-41.0)	25.0 (16.0-43.0)	29.0 (20.0-51.0)	<0.001[¶]
6. Albumin	46.8±2.8	46.8±2.9	45.5±3.2	0.116[§]
7. Diabetes mellitus				0.001[€]
Yes	22 (10.5%)	143 (14.5%)	134 (19.7%)	
No	185 (88.5%)	837 (85.1%)	543 (80.0%)	
8. Smoking				<0.001[€]
Non-smoker	135 (64.6%)	503 (51.1%)	250 (36.8%)	
Former smoker	59 (28.2%)	371 (37.7%)	269 (39.6%)	
Current smoker	14 (6.7%)	104 (10.6%)	152 (22.4%)	
9. Alcohol				<0.001[€]
Never/occasional drinker	142 (68.0%)	554 (56.3%)	143 (48.8%)	
Former drinker	24 (11.5%)	173 (17.6%)	122 (18.0%)	
Current drinker	42 (20.1%)	250 (25.4%)	221 (32.5%)	
10. Plaque index	50.0 (32.1-66.6)	59.0 (41.0-75.0)	73.0 (56.2-85.7)	<0.001[¶]

Abbreviations: BMI = body mass index, SBP= systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein, LDL = low density lipoprotein, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase. [$] Differences in the number of subjects are due to missing data. [€] The association between periodontitis groups and other categorical variables, analyzed using Chi-square test. [¶] The comparison between periodontitis groups, analyzed using Kruskal-Wallis H test. [§] The comparison between periodontitis groups, analyzed using ANOVA test. * Significant differences between groups at $P < 0.05$, analyzed using Tukey's post-hoc test analysis. ** Significant differences between groups at $P < 0.005$, analyzed using Mann-Whitney U test.

The median sST2 concentration of subjects was 18.1 (IQR:14.3-22.5) ng/ml. The concentration was 18.8 (14.9-23.3) ng/ml in male and 15.7 (12.9-19.8) ng/ml in female, in which the difference was statistically significant ($P < 0.001$) (Table 4). Moreover, the sST2 level was higher in subjects with diabetes mellitus than in those without disease ($P < 0.001$). In pairwise analysis, differences in sST2 levels were observed between subjects with severe chronic periodontitis and those with no/mild chronic periodontitis ($P = 0.002$). Moreover, the sST2 levels were higher in former smokers when compared to non-smokers ($P < 0.001$) and were higher in current drinkers when compared to non/occasional drinkers ($P < 0.001$).

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Table 4 Serum sST2 levels in relation to clinical parameters.

Variable	sST2 levels (Median(IQR))		P value
1. Sex : Male	18.8 (14.9-23.3)		<0.001†
: Female	15.7 (12.9-19.8)		
2. Diabetes mellitus : Yes	19.6 (15.6-24.7)		<0.001†
: No	17.8 (14.2-22.0)		
3. Periodontitis : No/mild	17.0 (13.2-21.3)	*	0.01¶
: Moderate	18.1 (14.3-22.6)		
: Severe	18.5 (14.7-23.0)		
4. Smoking : Non-smoker	17.3 (14.0-21.6)	*	0.002¶
: Former smoker	19.1 (15.0-23.4)		
: Current smoker	18.1 (14.6-23.2)		
5. Alcohol : Never/ occasional drinker	17.5 (13.2-21.7)	*	<0.001¶
: Former drinker	17.3 (14.3-21.5)		
: Current drinker	19.0 (14.8-23.7)		

¶ The data was analyzed by Kruskal-Wallis H test. † The data was analyzed by Mann-Whitney U test. * Significant difference between groups at P < 0.005, analyzed using Mann-Whitney U test.

Univariate linear regression analysis showed that sST2 levels were associated with sex, GGT, AST, albumin, diabetes mellitus, triglycerides, LDL, former and current smoker, current drinker, plaque score, and moderate and severe chronic periodontitis (P < 0.05) (Table 5). According to multivariate linear regression model, sex, GGT, AST, albumin, diabetes mellitus and triglycerides were significantly associated with sST2 levels (P < 0.05).

Table 5 Linear regression models estimating the association between sST2 and independent variables.

Variable	Univariable*			Multivariable†		
	Regression coefficient	SE	P value	Regression coefficient	SE	P value
Sex	-3.146	0.484	<0.001	-2.214	0.455	<0.001
GGT	0.042	0.003	<0.001	0.028	0.003	<0.001
AST	0.145	0.010	<0.001	0.107	0.014	<0.001
Albumin	-0.421	0.068	<0.001	-0.292	0.064	<0.001
Diabetes mellitus	3.102	0.569	<0.001	2.012	0.534	<0.001
Triglycerides	0.007	0.002	0.001	-0.008	0.003	0.004
HDL	-0.003	0.014	0.822	-	-	-
LDL	-0.019	0.005	<0.001	-	-	-
Cholesterol	-0.013	0.005	0.007	-	-	-
Smoking						
-Former smoker	1.916	0.455	<0.001	-	-	-
-Current smoker	1.401	0.627	0.026	-	-	-
Alcohol						
-Former drinker	0.117	0.579	0.841	-	-	-
-Current drinker	1.738	0.488	<0.001	-	-	-
Plaque score	0.028	0.009	0.002	-	-	-
Periodontitis						
- Moderate	1.611	0.690	0.020	-	-	-
- Severe	2.164	0.717	0.003	-	-	-

Abbreviations: SE = standard error, GGT = gamma-glutamyl transferase, AST = aspartate aminotransferase, HDL = high density lipoprotein, LDL = low density lipoprotein. * Linear regression analysis of each variable individually. † Multivariate linear regression analysis, building by backward method.

Because sST2 levels were much higher in male than in female, multivariate linear regression models were built separately for male and female. The result showed that factors influencing sST2 levels differed between sexes. In male, sST2 was associated

with GGT, AST, albumin, diabetes mellitus and triglycerides (Table 6). In female, the concentration of sST2 was associated with AST, albumin, diabetes mellitus, HDL, and moderate and severe chronic periodontitis.

Table 6 Multivariate linear regression models estimating the association between sST2 and independent variables, categorized by sex[†].

Variable	Male			Female		
	Regression coefficient	SE	P value	Regression coefficient	SE	P value
GGT	0.028	0.004	<0.001	-	-	-
AST	0.117	0.016	<0.001	0.066	0.022	0.003
Albumin	-0.307	0.082	<0.001	-0.220	0.086	0.011
Diabetes mellitus	2.032	0.639	0.002	2.047	0.873	0.019
Triglycerides	-0.009	0.003	0.014	-	-	-
HDL	-	-	-	0.040	0.016	0.011
CP1	-	-	-	1.514	0.669	0.024
CP2	-	-	-	2.054	0.845	0.015

Abbreviations: SE = standard error, GGT = gamma-glutamyl transferase, AST = aspartate aminotransferase, HDL = high density lipoprotein, CP1 = moderate chronic periodontitis, CP2 = severe chronic periodontitis. [†] Multivariate linear regression analysis, building by backward method.

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CHAPTER V

DISCUSSION AND CONCLUSION

To our knowledge, this is the first community-based study of the association between chronic periodontitis and sST2. The results showed that sST2 concentrations were associated with sex, GGT, AST, albumin, diabetes mellitus, and triglycerides. The subgroup analysis by sex demonstrated that sST2 levels were associated with GGT, AST, albumin, diabetes mellitus and triglycerides in male, but were associated with AST, albumin, diabetes mellitus, HDL, and moderate and severe chronic periodontitis in female.

Our study found that sST2 concentrations were higher in men than in women. This finding is in agreement with previous studies (Chanyavanich et al., 2014, Coglianese et al., 2012). Several studies have found that sST2 concentrations are varied among individuals and its levels are influenced by age, sex and race (Chanyavanich et al., 2014, Coglianese et al., 2012). A possible explanation of this observation is a genetic factor which may account for approximately 40% of the variation in sST2 levels (Ho et al., 2013). Moreover, the researchers hypothesized that the difference in sST2 levels between sexes may be due to differences in sex hormone. A large population-based study showed that female who received hormone replacement therapy had lower sST2 levels than those without hormone replacement therapy (Coglianese et al., 2012). In contrast, another study of healthy blood donors reported that there was no association between the levels of androgen or estrogen and sST2 levels in both genders (Dieplinger et al., 2011). Therefore, biological explanation for the variation of sST2 between sexes is still inconclusive.

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In univariate model, we found that sST2 levels were associated with former and current smokers and current drinkers. However, in multivariate model, these associations were not found after adjusting for other variables. The association between these health behaviors and sST2 seems to be influenced by sex. We found that more than ninety five percent of smokers and current drinkers were male. Because sST2 levels were higher in male than female, this may be a reason that smokers and current drinkers had an association with sST2 in univariate model. Moreover, we found that former smokers had higher levels of sST2 when compared to current smokers. We observed that 18.2 percent of former smokers were diabetics as compared to 14.8 percent in current smokers and 14.5 percent in non-smokers. In multivariate model, diabetes mellitus is one of the major factors associated with increased levels of sST2. This could explain why former smokers had higher levels of sST2 than the other two groups.

We also found that sST2 levels were associated with GGT, AST, albumin, diabetes mellitus and triglycerides in our study population. The GGT and AST are well-known liver enzymes that are used to evaluate liver function. They are also found in cell membrane of many tissues such as kidney, intestine and heart. Both GGT and AST have been used to predict the risk of mortality and cardiovascular events (Mason et al., 2010, Shen et al., 2015). Moreover, GGT might involve in atheroma formation (Emdin et al., 2005). In addition, a recent study in China found that sST2 levels in heart failure patients were associated with abnormal liver function and low levels of albumin (Zhang et al., 2014). Triglycerides and diabetes mellitus are traditional risk factors of CVDs and have been used to predict the CVDs risk (Khot et al., 2003). Moreover, the association between sST2 and diabetes mellitus has been shown in several studies (Coglianese et al., 2012, Chanyavanich et al., 2014). Therefore, the association of sST2 with diabetes

mellitus, triglycerides and enzymes that used to evaluate CVDs risk suggests that sST2 levels may be used as an indicator of cardiometabolic syndrome in this population.

In this study, the association between sST2 and chronic periodontitis was found only in female subjects. However, the mechanism linking between sST2 and chronic periodontitis is not clear. An *in vitro* study showed sST2 mRNA was elevated in periodontal tissues from chronic periodontitis subjects when compared with tissues from healthy periodontium (Malcolm et al., 2015). Moreover, sST2 mRNA expression was upregulated in a cultured gingival cell line after stimulated with *P. gingivalis* (Awang, 2014). In addition, in healthy subjects who were administrated with LPS, inflammatory cytokines, including IL-6 and TNF-α were elevated in 4 hours, followed by a massive augmentation of sST2 secretion in 24 hours (Mildner et al., 2010). Therefore, it is possible that periodontal pathogens or cytokines released from tissues with chronic periodontitis may lead to an elevated level of serum sST2.

Previous epidemiological studies indicate that sex may be an effect modifier for the association between chronic periodontitis and CVDs. Three studies in US, Korean, and German populations observed that the associations were stronger in men than women (Grau et al., 2004, Sim et al., 2008, Xu and Lu, 2011), whereas another study in US population reported a stronger association in women (Andriankaja et al., 2007). The reason for this sex-specific difference is still unknown. Our result showed that chronic periodontitis was significantly associated with sST2 levels, a biomarker for CVDs, only in female subjects. It is possible that women and men may differ in their inflammatory response to chronic periodontitis. In animal model, female rats with experimental periodontitis had higher serum concentrations of inflammatory cytokines including IL-1, TNF-α, and CRP, compared to male rats with periodontitis (Bain et al., 2009). Similarly, a human study with endotoxin challenge found that women had a more pronounced

inflammatory response to LPS than men as indicated by a greater increase in serum levels of IL-1, TNF-α, and CRP (van Eijk et al., 2007). Owing to the fact that sST2 was secreted in response to systemic inflammation, we hypothesize that female subjects with chronic periodontitis have higher levels of serum inflammatory cytokines, which in turn lead to elevated sST2 levels compared to male subjects with periodontitis. It should be noted that GGT and triglycerides which were the strong predictors of sST2 levels in male subjects were not significantly associated with sST2 in females. In contrast, HDL was significantly associated with sST2 in females, but not in males. Therefore, we proposed that there are sex differences in the predictors of serum sST2 levels, and these differences may help explain the sex-specific association between chronic periodontitis and CVDs.

One of the limitations of this study was that the study participants were not randomly selected, thereby may not be representative of the Thai population. However, the EGAT enterprise was composed of the individuals with a wide range of socio-demographic backgrounds (Vathesatogkit et al., 2012). Therefore, these study subjects represented a diverse group of the Thai population. Moreover, the periodontal examination in this study was a half-mouth examination using six sites per tooth. A question has been raise whether or not the half mouth examination may produce biases for severity and extent of disease estimates as compared to the full mouth examination. However, a recent systematic review showed that the half mouth analysis based on six-sites examination had a high sensitivity for prevalence estimates and a low bias for severity and extent of disease estimates, as compared to full-mouth three-sites examination (Tran et al., 2013).

In conclusion, the result of this study showed that sST2 levels were associated with sex, GGT, AST, albumin, diabetes mellitus and triglycerides. Moreover, the association between sST2 and chronic periodontitis was shown only in female subjects.

However, the mechanism linking between sST2 and chronic periodontitis is still unknown. Therefore, the association between sST2 and chronic periodontitis requires further investigation in both clinical and community-based studies for a better understanding of their association.

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APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
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VITA

Miss Dissayawadee Katudat was born on August 20, 1988 in Bangkok, Thailand. In 2012, she earned her Doctor of Dental Surgery degree from the faculty of Dentistry, Thammasat University. She worked as a general dentist at Sena Hospital, Phra Nakhon Si Ayutthaya (2012-2013) and Srithanya Hospital, Nonthaburi (2013-2014). Presently, she attends the Master of Science Program in Periodontics, Department of Periodontology, Faculty of Dentistry, Chulalongkorn University.

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