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Salivary and Serum Adiponectin and CRP Levels in Acute Myocardial Infarction Related to BMI and Oral Health

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Abstract

Objective—Adiponectin is produced by adipose cells and is considered an anti-inflammatory molecule. In contrast, C-reactive protein has been identified as a hallmark of systemic inflammation and used as a risk marker of cardiovascular disease. Of interest was the relationship of these two biomarkers to oral health and cardiovascular risk.

Methods—This investigation examined these two molecules in serum and unstimulated whole saliva of patients within 48 hrs. of an acute myocardial infarction (AMI) compared to control subjects. We hypothesized a differential response in these biomolecules resulting from the heart attack that would be affected by both the BMI and oral health characteristics of the individuals.

Results—Significantly lower adiponectin levels were observed in the serum of the AMI patients. Serum adiponectin in both groups and salivary adiponectin in AMI patients decreased with increasing BMI. Oral health was significantly worse in the AMI patients, and both serum and salivary adiponectin were elevated with better oral health in control subjects. Serum CRP levels were increased in the AMI patients regardless of their oral health, and both serum and salivary CRP were significantly elevated in S-T wave elevated MI patients (STEMI).

Conclusions—These initial data provide evidence relating obesity and oral health to salivary and serum analyte levels that occur in association with cardiac events. Relationships have been described between cardiovascular (CVD) risk and periodontal disease. Additionally, various systemic inflammatory biomarkers appear to reflect both the CVD risk and the extent/severity of

periodontitis. Our findings indicated that oral health and obesity contribute to altering levels of these salivary and serum analytes in association with cardiac events. The potential that serum and/or salivary biomarkers could aid in evaluating CVD risk requires knowledge regarding how the oral health of the individual would impact the effectiveness of these biological measures.

Keywords

myocardial infarction; saliva; adiponectin; CRP; BMI; oral health

INTRODUCTION

Adiponectin is a circulating plasma protein produced abundantly and specifically by adipocytes and is detected at relatively high total levels in the bloodstream of humans that regulates the metabolism of lipids and glucose (1–4). Adiponectin is a major adipocyte-derived protein with anti-inflammatory and anti-atherogenic properties affecting endothelial cells, thus appearing to play a protective role in atherosclerosis development and progression (5, 6). C-reactive protein (CRP) is released systemically as part of the acute phase response to active inflammation including infection, injury, neoplasia, or chronic local inflammatory conditions, *e.g.* arthritis. Elevated CRP levels have been associated with an increased risk of heart attack and support the contribution of chronic inflammation to atherosclerotic vascular changes and coronary risk (7, 8). Recent findings suggest that elevated CRP may be just as important as elevated LDL cholesterol levels in predicting CVD risk, and that high CRP levels may identify high-risk patients (9). Thus, CRP could be considered an independent marker of cardiovascular risk (10, 11). These two mediators in serum appear to provide information regarding anti-inflammatory (*e.g.* adiponectin) and pro-inflammatory (*e.g.* CRP) responses that could link metabolic syndrome, obesity, diabetes and cardiovascular disease.

Various studies have begun evaluating an array of biomolecules in saliva as a potential diagnostic fluid for both oral and systemic diseases (12–14). Numerous reports have explored salivary profiles of analytes related to both oral (14–17) and systemic diseases, including metabolic syndrome, diabetes, atherosclerosis and myocardial infarction (12–14, 18, 19), with some of the studies including an assessment of oral disease. Furthermore, a fundamental basis in consideration of utilization of saliva as a diagnostic fluid is some consistency/stability in alterations in levels of targeted analytes between health and disease (14, 20, 21). In this regard, various reports have described this type of differentiation in health and disease, and stability of selected analytes, similar to those evaluated in the current study (22, 23). Kosaka et al. (24) recently demonstrated positive correlations of salivary cytokines including IL-6, TNF α and PGE $_2$ with Odds Ratios of 2–3 for carotid atherosclerosis. Due to its important cardio-metabolic actions, adiponectin represents a biological molecule of interest and is a potential emerging biomarker of disease. Furthermore, CRP levels in serum and saliva have been identified related to CVD (25–28). Thus, we targeted these two biomolecules in various biological fluids of patients suffering myocardial infarction that should enable additional information on their relationship to these clinical events and oral health of the individuals.

MATERIALS and METHODS

Patient Recruitment

The rights of human subjects involved in this study were protected by the institutional review board of the research site. In all cases, informed consent was granted prior to sample collection. To ensure privacy rights of study participants, all samples were tested in a de-identified manner.

A cross-sectional clinical study was implemented, and 92 patients were recruited with acute myocardial infarction (AMI), along with 111 age- and gender-matched non-AMI controls at hospitals of the University of Kentucky and University of Louisville. All AMI patients were enrolled within 48 hours of their cardiac event. Patients were classified as STEMI based on ECG elevation of ST-segments by -0.1 mV in contiguous leads in patients with ischemic symptoms and increased cardiac biomarkers (99th percentile of the upper reference limit for troponin I (TnI), cutoff 0.04 ng/mL). Diagnoses of NSTEMI were made for patients with ischemic symptoms and ECG changes consistent with ischemia (depression of the ST segments or new left bundle branch block), new pathologic Q-waves, or evidence of perfusion defects on stress test, and were followed by confirmatory positive TnI test as a standard measure of myocardial damage. All study participants were at least 18 years old. Exclusion criteria were fever, stroke, immune disorders, use of steroid medications, organ complications/failure, and inability to provide saliva. Demographic information was obtained (Table 1), medical records reviewed, oral evaluation performed, and biological fluids obtained (blood and UWS) from each study participant. Samples were transported on ice to a local laboratory, centrifuged, and divided into aliquots. The specimens were generally kept on ice for <1 hr., prior to being stored at -80°C , and analyzed within 3 months of collection.

As we have described previously, oral health was assessed visually at the bedside to not interfere with medical management of the AMI patients or in the dental operator for controls. Oral health was scored as poor, fair, or good based similar to approaches used previously (29, 30). Poor was defined as generalized areas of mucosal inflammation, multiple broken down teeth, obvious tooth or gum infection or reports loose teeth; Fair was defined as may have oral complaints, localized areas of mucosal inflammation, areas of visible decay, no obvious tooth or gum infection; and Good was defined as no complaints, no obvious mucosal inflammation, no reported loose teeth or symptoms of disease. Additionally, the number of teeth was determined as an estimate of past and existing dental disease (31). Informed consent was granted prior to sample collection. To ensure privacy rights of study participants, all samples were de-identified and then assayed.

Sample Collection and Processing for Analysis of Serum and Salivary Analytes

Two vacutainer tubes of blood were collected from each subject. One tube was sent to the University of Kentucky Chandler Medical Center Hospital CLIA-approved laboratory for assessment of serum lipid levels. The second tube was processed for serum collection, aliquoted and stored frozen at -80°C . Unstimulated whole expectorated saliva (UWS) samples (5 mL) were collected from each subject at baseline and at 4 (\pm 30 min.) hours

post-procedure according to a modification in the method described by Navazesh (32). Samples were collected into sterile tubes containing a protease inhibitor solution (SIGMAFAST, Sigma, St. Louis, MO.), then transported to the laboratory on ice, centrifuged, separated into aliquots and stored at -80°C until analyzed.

A Luminex IS-100 instrument was used for multiplexed detection of C-reactive protein (CRP) and adiponectin with kits available from Beadlyte Technology (Millipore, St. Charles, MO, USA) (13). All samples were analyzed in duplicate. Standards were included on all runs and results are reported within the linearity of the assays. The adiponectin assay range was 250-0.08 ng/mL with a minimal detectable dose (MDD) of 0.056 ng/mL and the CRP assessment was 50-0.016 ng/mL with an MDD of 0.0012 ng/nL. These two host response molecules are studied in the context of serum and unstimulated whole saliva for patients with acute myocardial infarction (AMI) and compared to age and gender matched non-AMI control subjects.

Blood lipid levels were determined using a Beckman UniCel DxC 800 Synchron Clinical Systems instrument in the Clinical Laboratory Improvement Amendments (CLIA)-certified serum chemistry laboratory of the University of Kentucky Hospital.

Statistical Analysis

Interval level demographic variables were presented as mean and standard deviation (SD) and categorical demographic variables as frequencies and percentages. Biomarker levels were presented as mean, median and SD. Percentages were compared among groups using chi-square statistics. Comparison of means were based on one way ANOVA, two way ANOVA or two-sample t-tests depending on the number of groups being compared; each biomarker was log transformed in these comparisons. Pearson's correlations were based on log transformed biomarker data. Statistical analyses were performed using the PC SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with significance determined at $p < 0.05$ level.

RESULTS

Demographics of population

Table 1 shows that the control subjects were slightly younger and more likely to be female, but were comparable to AMI patients on percent white race, and percent reporting current alcohol use. As expected the NSTEMI and STEMI groups had significantly more tobacco users than controls. Table 1 also shows that the AMI and control groups are comparable on mean BMI, and triglycerides levels, but that the control group had elevated total cholesterol, LDL and HDL levels compared to the AMI group ($P < 0.004$ in each case).

Adiponectin and CRP levels in AMI

Figure 1A depicts the levels of adiponectin and CRP in serum and saliva from the AMI and control groups. As might be expected, CRP levels were significantly elevated in both serum and saliva of the AMI patients ($P < 0.0001$, in each case) (8). As expected based upon the vascular protective effects of adiponectin, significantly increased levels in the serum of control group compared to the AMI patients were observed ($P = 0.0034$). In contrast, the

adiponectin levels were increased by about 50% in saliva from the AMI patients, although this did not reach statistical significance ($P = 0.58$).

Based on our previous data (13) suggesting variations in levels of selected salivary analytes in AMI patients presenting with STEMI compared to NSTEMI coronary events, we stratified the AMI patients into these subsets. Here significantly increased levels of CRP in both serum and saliva of STEMI and NSTEMI patients compared to controls ($P < 0.0001$ in all cases) were observed (Figure 1B). In addition, the STEMI patients showed significantly higher CRP concentrations compared to NSTEMI patients ($P = 0.0035$ in both cases). A similar overview for serum adiponectin levels showed a stepwise decrease in adiponectin levels from control subjects through STEMI patients ($P = 0.0035$, control versus STEMI and $P = 0.082$ control versus NSTEMI). In contrast, saliva adiponectin levels were not significantly different among these three groups ($P = 0.62$).

BMI and lipids related to adiponectin and CRP

Figure 2 explores the relationship between BMI and levels of serum and salivary adiponectin and CRP. Serum adiponectin levels were elevated in the controls compared to AMI in BMI tertiles 1 and 3, but not tertile 2 ($P = 0.0075$, 0.013 , and 0.83 , respectively). In contrast, saliva adiponectin did not vary systematically between control and AMI in any tertile, although the control levels did decline with tertile. On the other hand, the levels of CRP were increased monotonically in AMI compared to control in all tertiles for both serum and saliva ($P < 0.003$ in all cases) and in serum appeared to vary by tertile in both groups.

Table 2 summarizes the observed relationships between the serum and salivary analytes, and the serum lipid levels. Adiponectin levels in serum and saliva were positively correlated with serum HDL and negatively correlated with serum triglyceride levels in the control subjects ($P < 0.04$ for each comparison). In the AMI patients, salivary adiponectin levels were positively correlated with total cholesterol and LDL levels ($P < 0.001$ in both cases). With CRP, significant relationships were not noted in either biological fluid in either of the cohorts. Levels of both adiponectin and CRP in the two biological fluids were significantly positively correlated in the control subjects ($P < 0.008$ in both cases) and were positively correlated for adiponectin in the AMI patients ($P = 0.008$).

Oral health relationship to adiponectin and CRP

Figure 3 summarizes the comparison of oral health in the control subjects and AMI patients. The AMI patients had significantly fewer teeth and significantly worse overall oral health than the control group ($P < 0.0001$ for each comparison). The relationship of serum and salivary adiponectin and CRP with the number of teeth in each patient was examined in the context of the severity of oral disease as shown in Figure 4. This data reveals significantly increasing levels of serum CRP in AMI subjects with increasing number of teeth. Serum CRP levels were significantly elevated in AMI patients compared to controls irrespective of the number of teeth present ($P < 0.03$ for each subgroup). Salivary CRP was also increased in the AMI versus control subjects in the lowest and highest subgroups ($P < 0.008$ in both cases but not the middle subgroup). Figure 4 also depicts adiponectin levels. In serum, control levels of adiponectin are elevated for the two subgroups with the most teeth ($P < 0.02$ in both

comparisons) while salivary adiponectin levels were elevated in the AMI group compared to the controls in only the highest subgroup ($P=0.02$).

DISCUSSION

The BMI levels were similar in the AMI and control groups, which may be expected, since the control patients were selected from family and acquaintances of the patients with the cardiac events enlisted into the study. However, the AMI patients had significantly fewer teeth and significantly worse oral health than the control group, consistent with some of the existing literature supporting worse oral health in patients with cardiovascular diseases and the potential linkage of chronic infection and inflammation in the oral cavity contributing to systemic risk changes for CVD (33–37).

Consistent with the documented elevation in CRP levels in patients at risk for CVD events (9), the levels were significantly elevated in both serum and saliva of the AMI patients. Of particular interest, was the observation that even using a control population with a number of existing risk factors for CVD, CRP was significantly elevated above the controls in both fluids from the patients who had a cardiac event. These findings were even more pronounced with levels of CRP in serum and saliva of STEMI and NSTEMI patients compared to controls, with STEMI patients showing the greatest levels of CRP. This finding is consistent with the STEMI patients generally considered to have a more severe myocardial infarction and tissue damage (38). Since the samples were collected within 48 hrs. of the event, we cannot determine whether these elevated CRP levels presaged the AMI or if this molecule increased rapidly post-ischemia, or both, and is a limitation of this investigation.

Adiponectin is considered to exert anti-inflammatory effects via macrophages through suppressing the production of pro-inflammatory cytokines (1) and contributes to a significant reduction of the risk of myocardial infarction, even after correction for HDL- and LDL-cholesterol and body mass index (BMI) (39). In non-diabetic subjects, levels of adiponectin are inversely related to CVD severity (4) and low plasma adiponectin is associated with an early onset of coronary heart disease emphasizing that adiponectin is influenced by body fat status and associated with CVD (40). Adiponectin has also been reported to be negatively correlated with plasma triglycerides and positively correlated with HDL (3, 5). The present study demonstrated that adiponectin levels were significantly increased in the serum of control versus AMI patients, which might be expected from the existing literature. Moreover, the serum adiponectin levels were lowest in the STEMI patients, consistent with a lower level of this protective adipokine in patients with the most severe cardiac events.

The adiponectin and CRP levels were inversely related within these populations, similar to a report by George and coworkers (41) showing a negative relationship of serum adiponectin and CRP levels, reflecting an increased risk indicator of cardiovascular disease. Plasma adiponectin levels are decreased in obesity (39, 42, 43). We observed lower levels of serum adiponectin and higher levels of CRP in both control subjects and the AMI patients related to BMI of the individuals. This is consistent with an increased level of obesity coincident with decreased systemic adiponectin, which would represent an anti-inflammatory protective

factor for CVD in the patients. Moreover, the pro-inflammatory aspects of excess adipose tissue would be expected to contribute to adverse levels of CRP in the serum that has been demonstrated as a predictive risk indicator for CVD (9, 11, 44). An accompanying interesting interpretation of these findings was that this relationship existed in both the control subjects and AMI patients. As noted previously, we deliberately selected a control population, as not inherently CVD normal, but one that was similar to the AMI patients in demographic and clinical features, absent a cardiac event. The results then would imply that a number of the “control” patients demonstrated multiple risk markers for a future ischemic event.

Some previous studies have identified adiponectin in saliva (45, 46) and provided evidence validating the characteristics of the adiponectin biomolecules in saliva (47). Toda et al. (45) found a significant correlation between salivary and serum adiponectin in healthy male volunteers >43 years of age. A more recent study confirmed this relationship in both genders, as well as younger subjects (48). We observed that salivary adiponectin levels decreased in the AMI patients with increased BMI. Thus, there appears to be a reflection of the host responses to obesity that is reflected in both the serum and saliva of the subjects, albeit, the existence of a cardiac event modulates these responses. Nigro et al. (47) recently evaluated adiponectin level in saliva related to obesity and show a higher expression of both high and low molecular weight forms in saliva from obese patients, suggesting a potential for salivary biomarkers in metabolic disorders. Elevated salivary CRP levels (6-fold) and decreased adiponectin levels (30%) were noted in obese children (49). Saliva and serum adiponectin levels were correlated and plasma adiponectin was decreased with increased triglyceride levels and waist circumference in metabolic syndrome patients (50). as well as correlations in salivary and serum CRP and adiponectin in postmenopausal women (51).

The adipokines (resistin, visfatin, and adiponectin) were examined in saliva of healthy individuals and showed a significant correlation between adiponectin in saliva and serum from healthy subjects, but provided no insight into variations in this biomolecule related to disease, or risk of disease (48). In the present study, both adiponectin and CRP levels were significantly positively correlated in the biological fluids from the control subjects suggesting that the salivary levels of the molecules likely are reflecting a direct serum contribution to the oral cavity irrespective of the level of oral health or disease. Interestingly, this relationship was lost in the AMI patients.

A study identified a substantial increase in periodontitis in AMI patients, associated with elevations in serum CRP, but not adiponectin (52). In the PAROKRANK Study (Periodontitis and Its Relation to Coronary Artery Disease), in patients with an initial myocardial infarct MMP-8 and myeloperoxidase were elevated in non-MI versus MI patients and primarily correlated with clinical signs of periodontal inflammation (53). Salivary CRP levels have been shown to be correlated with arterial blood pressure, BMI, and intima-media thickness, as was MMP-9 in saliva. Lipid inflammatory mediators (LTB₄ PGE₂) in saliva were also associated with arterial stiffness (54). Salivary malondialdehyde levels (a measure of lipid peroxidation) were elevated in both chronic adult periodontitis and acute coronary syndrome patients. These levels also correlated with periodontal clinical

parameters and levels of serum CRP and fibrinogen (55). Furthermore, a recent case-control study identified significantly elevated salivary CRP levels in the periodontitis group (56).

Significantly increased levels of serum CRP in the control subjects was related to increasing numbers of teeth. In contrast, serum CRP levels were significantly elevated in AMI patients compared to controls irrespective of the number of teeth present. Control subjects with the fewest teeth had the lowest serum adiponectin levels and the AMI group presented a profile of adiponectin that was generally unaffected by the number of teeth present. Salivary adiponectin levels were generally increased in both controls and AMI patients with increasing number of teeth, while salivary CRP was only elevated in AMI patients with the greatest number of teeth. These findings indicate a relationship of the inflammatory (CRP) and anti-inflammatory (adiponectin) biomolecules with BMI, and an association of these levels that relate to tooth number and potentially reflect the level of oral health. Interestingly, studies have also demonstrated that oral bacterial stimulation of periodontal cells can induce local production of adiponectin (57). Moreover, administration of adiponectin in a murine periodontitis model reduced alveolar bone loss, reflecting altered osteoclastogenesis with decreased osteoclast numbers (58), thus supporting some relationship between these biomolecules and biological processes occurring in the oral cavity.

While a limitation of the study could be perceived to be related to the predominant Caucasian distribution of the population, the overall profile of our study group was in the range of the 75% U.S. and 90% Kentucky race/ethnicity population base. Moreover, a potential confounder in the clinical assessment of the subjects, was the potential that dental services were provided immediately preceding entry into the study and could affect these measures, as well as the potential that following AMI, general oral hygiene practices could be substantially altered leading to a readout of worse oral health. The clinical evaluation of the AMI patients was conducted within 48 hrs. of the event, thus, we feel it unlikely that this interval would have minimal impact on the tooth numbers and overall oral health categorization. Additionally, while it is possible that the control group had a greater number of individuals that had received recent dental prophylaxis versus the AMI patients, we accessed the control group from the general subject pool of individuals as represented by the AMI patients. Thus, we believe it is unlikely that the access to active professional dental services significantly impacted the overall oral health evaluation between the two groups, we cannot totally eliminate this confounder with the data available. These findings provide insight into the interrelationships of a systemic anti-inflammatory and proinflammatory biomarker in AMI patients, including documenting difference in levels of these analytes in unstimulated whole saliva that could contribute to profiling the biologic risk of individuals for cardiac events.

Acknowledgments

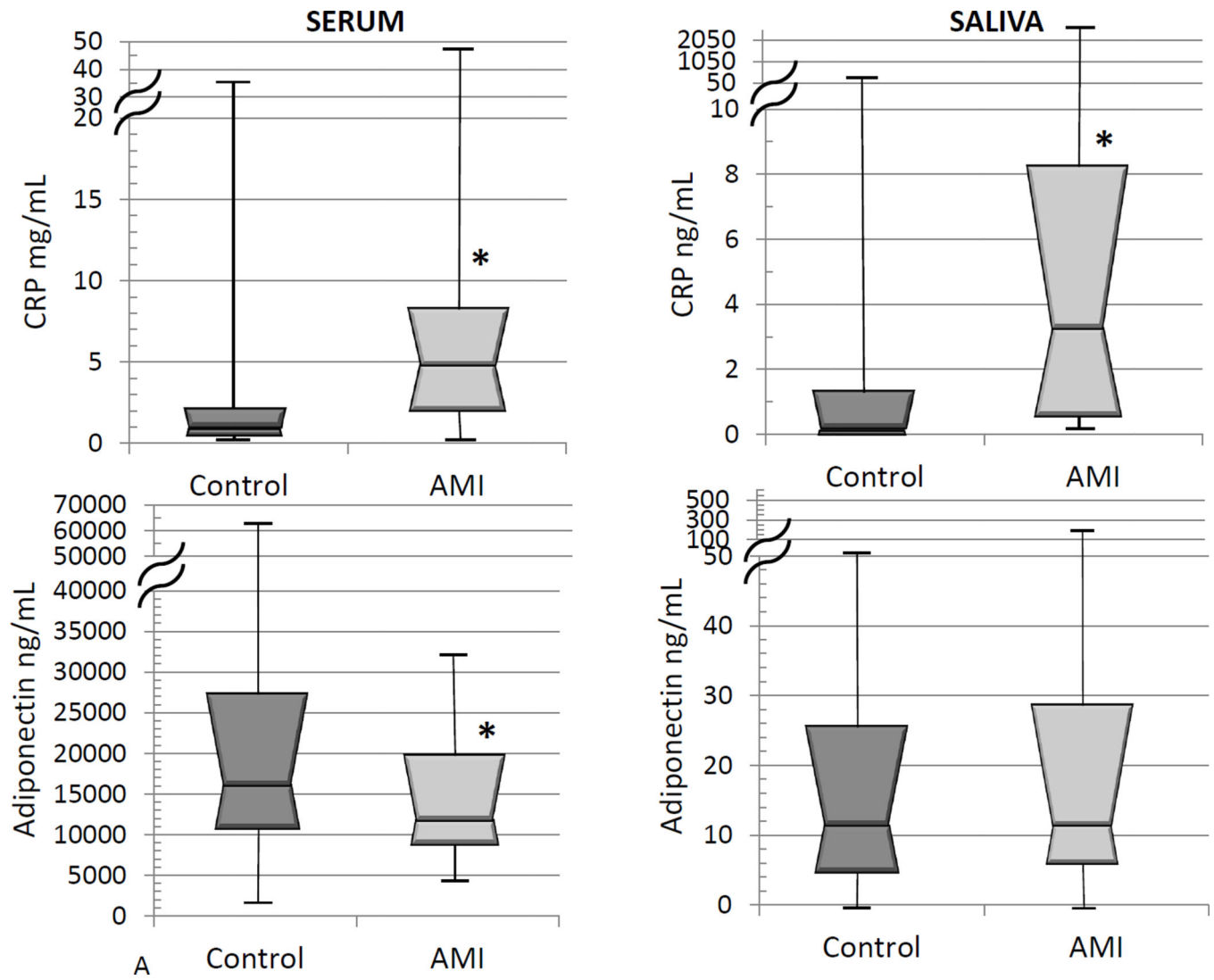
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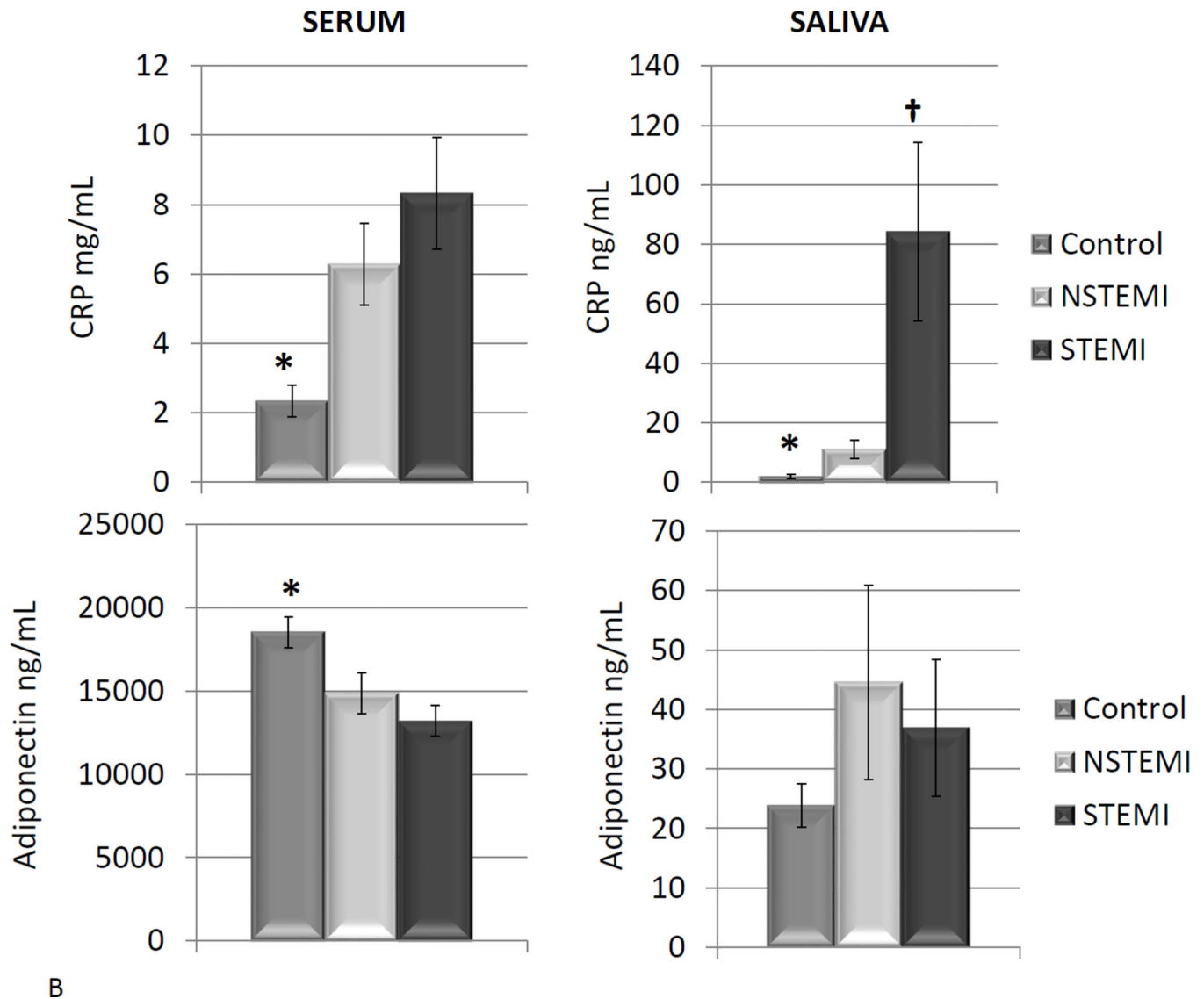


Figure 1.

(A) Box plot of levels of adiponectin and CRP in serum and saliva from the AMI and control groups. Box represents 25th–75th percentile, horizontal line is median value, and whiskers denote range of values in the groups. The asterisk (*) denotes significantly different from control group at least at $p < 0.05$. (B) Levels of CRP and adiponectin in serum and saliva of STEMI and NSTEMI patients compared to controls. The asterisk (*) denotes significantly different from disease groups at least at $p < 0.05$, and cross (†) denotes significant difference in levels in STEMI vs. NSTEMI at $p < 0.01$.

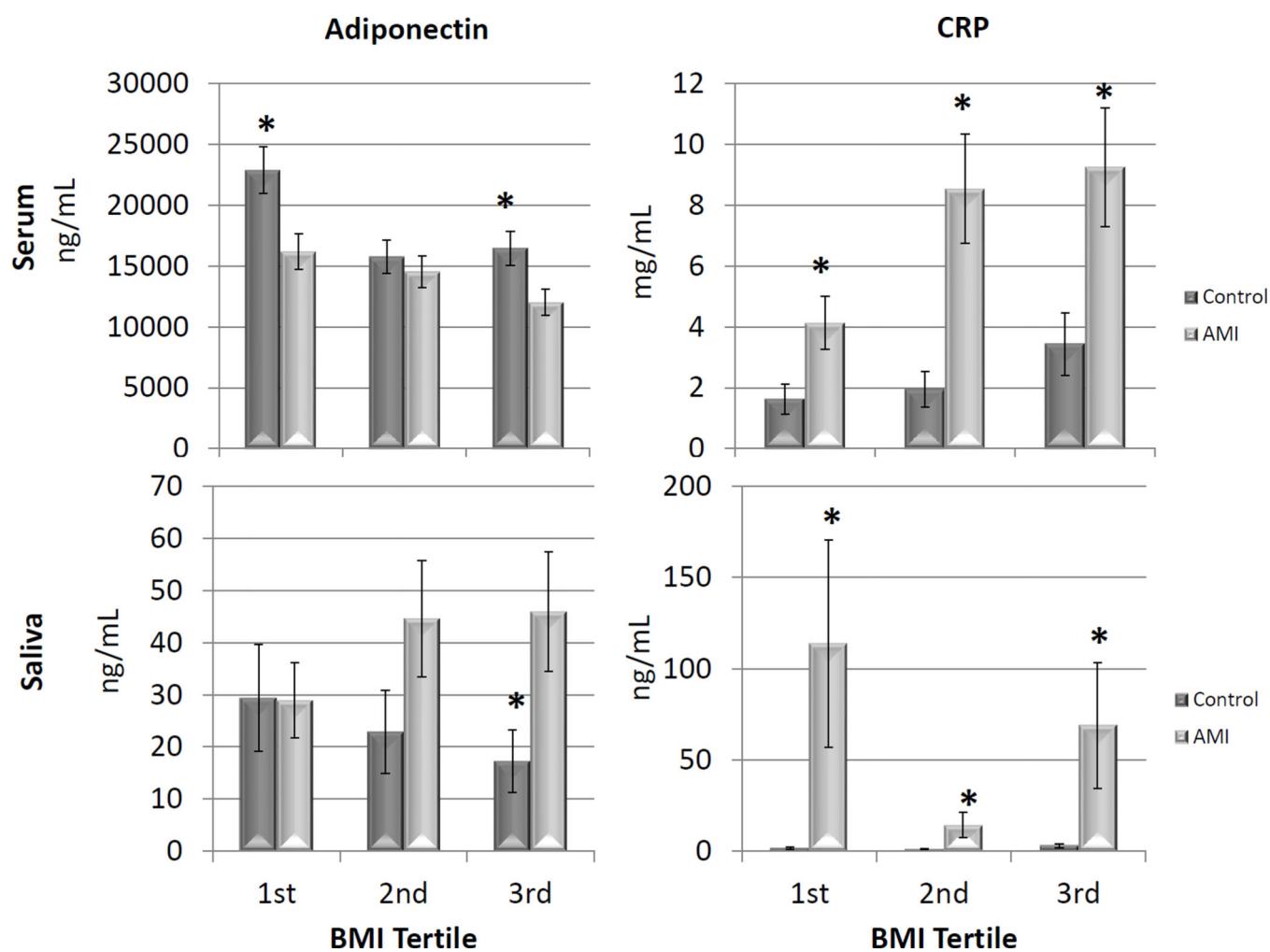


Figure 2.
Levels of serum and salivary adiponectin and CRP in both control subjects and AMI patients related to tertiles of BMI of the individuals. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk denotes levels in control significantly different from AMI at least at $p < 0.05$.

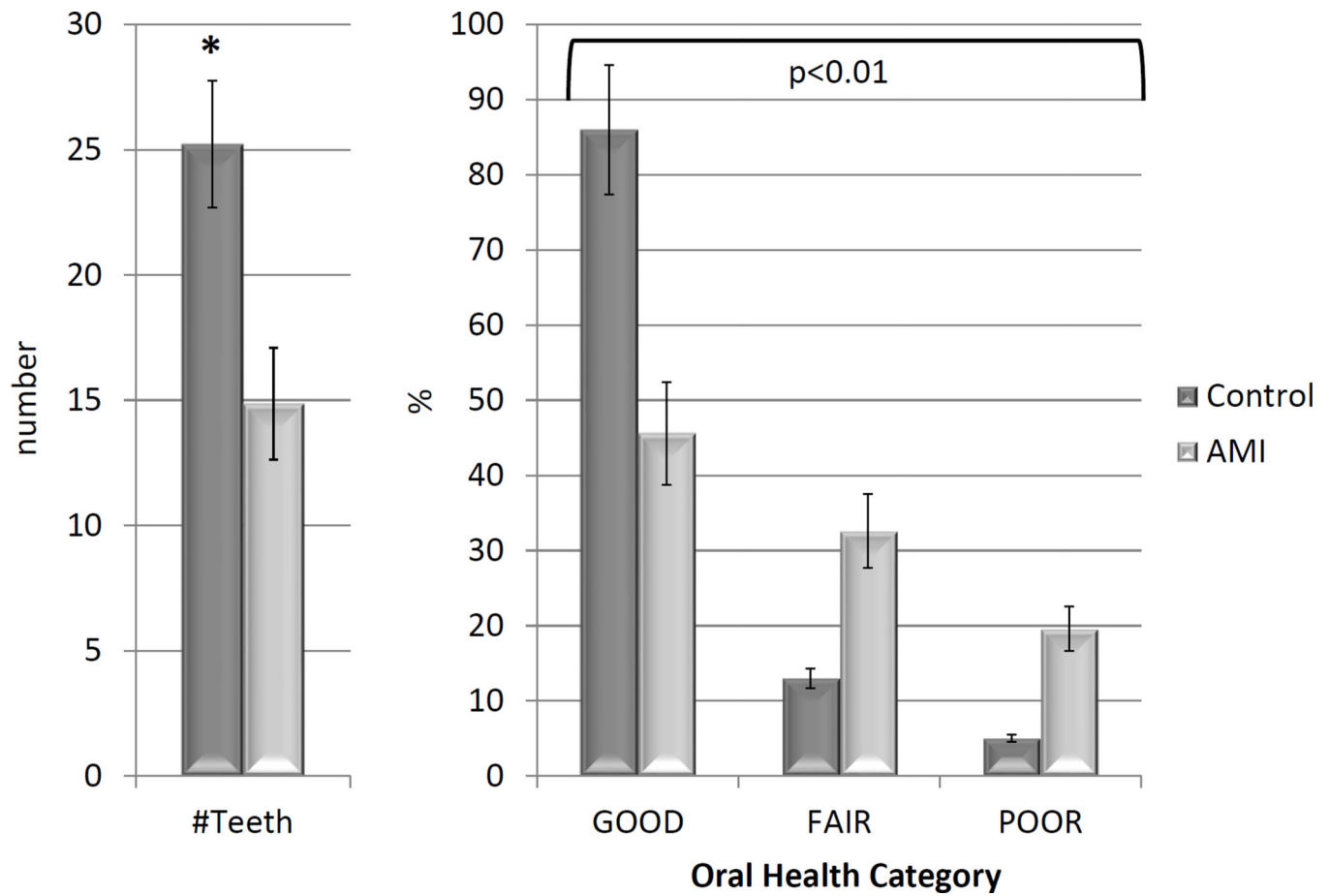


Figure 3. summarizes the comparison of oral health in the control subjects and AMI patients, both as number of teeth and oral health categorization. The asterisk denotes significant difference at $p < 0.05$.

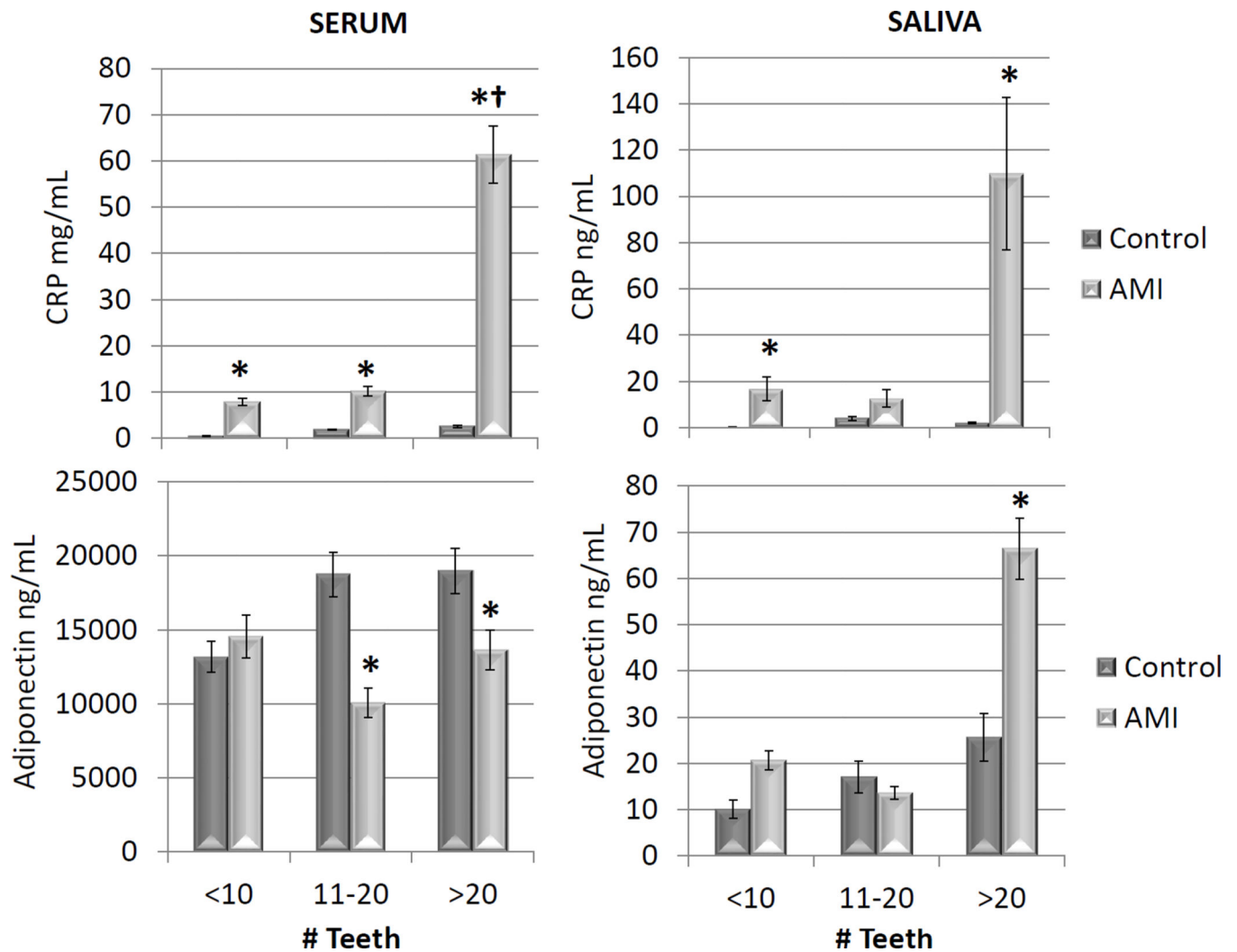


Figure 4.

Levels of serum and salivary CRP and adiponectin in control and AMI subjects with increasing numbers of teeth. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes significantly different from control group at $p < 0.05$. The cross (†) denotes significantly different from other AMI tooth categories at $p < 0.01$.

Table 1

Demographics, BMI and serum lipid levels in the control and AMI subjects.

		AMI		
	Control	NSTEMI	STEMI	TOTAL
N	111	34	58	92
Age (yrs.)	48.6 ± 8.9	53.1 ± 11.7	54.8 ± 12.0	54.2 ± 11.9
Female (%)	55.9	29.4	29.3	29.3
White (%)	84.7	82.4	86.2	84.8
Current Tobacco Use (%)	22.5	58.8 *	77.6 *	70.7 *
Current Alcohol Use (%)	26.1	26.5	32.8	30.4
Total Cholesterol	189.6 ± 40.1 *			148.1 ± 65.9
HDL	46.8 ± 13.1 *			39.8 ± 35.4
LDL	116.5 ± 29.8 *			93.3 ± 39.2
Triglycerides	151.1 ± 87.6			168.8 ± 102.5
BMI	28.1 ± 5.6			29.0 ± 6.3

* Denotes significantly different from control at p<0.05.

Table 2
Relationship of serum and salivary analytes to serum lipid levels in control subjects and AMI patients.

Analyte	Patient Pop.	Fluid	Chol	HDL	LDL	Trig	Ser vs. Sal
Adipo	Control	Serum	-0.0055	0.4238	0.1600	-0.3955	0.2695
		Saliva	0.1923	0.2794	0.1885	-0.1618	
	AMI	Serum	0.0632	-0.0322	0.1376	-0.1141	0.2947
		Saliva	0.4369	0.0055	0.5424	-0.0137	
CRP	Control	Serum	0.0165	-0.1996	0.0517	0.0298	0.4405
		Saliva	0.0281	-0.0555	-0.0230	0.0739	
	AMI	Serum	0.1262	-0.1484	0.1061	-0.1374	0.0643
		Saliva	0.0505	-0.0668	0.0915	-0.1175	

Note: Correlation values in **bold** significant at least at p<0.05