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INVITED EDITORIAL

Salivaomics in systemic disease: an evolving science- an interesting prospect for 'lab-on-chip' diagnostics in coronary artery and other systemic diseases

Sushila Sripad[1,2]

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Abstract This is an invited editorial to comment on the original article by Vasaghi-Gharamaleki et al. [1] published elsewhere in this issue. There are several components to that study that need attention—choice and validation of biomarkers in plasma/saliva in systemic disease like coronary artery disease (CAD), normal response to surgically induced stress, timing and duration of the exercise study, clinical profile of the CAD and risk assessment.

The editorial gives a brief background of the translational medicine/technology that developed as an offshoot of the Human Genome Project and includes a variety of Omics studies, of which salivaomics is one. Further, from the literature analysed, it would be clear that the study design, absence of data for clinical risk assessment, study of limited number of salivary biomarkers without parallel study of serum biomarkers and study restricted to 8 weeks in the presence of anthropometric data suggesting that the normal surgical stress response is ongoing—all point to major deficiencies and no valid conclusions can be drawn from the study.

The idea of creating a map of human genes dates back to the 1980s [2]. The Human Genome Project (HGP) 1990–2003 led to mapping of whole genome as well as to *sequencing and mapping of individual genes* and a spurt in biotechnology, bioengineering, *gene splicing and other applications*. This, in turn, led to a spurt in translational medicine. Technology for simultaneous measurements of an enormous array of biomarkers (multiplex arrays) in serum/plasma, urine, saliva, cerebrospinal fluid and several other body fluids increased manifold. A whole science of 'omics' has evolved since: Genomics, Proteomics, Metabolomics, Metallomics, Transcriptomics, Urinomics, Salivaomics, Bacteriomics, Viromics, Pharmacogenomics etc. Intense research on diseases at the molecular level transformed clinical medicine in the twenty-first century.

In a consensus study report, Micheel et al. [3] referred to the challenges of translational 'omics' that require development of 'rigorous statistical, bioinformatics, laboratory and clinical procedures' to validate the tests and evaluate their clinical usefulness. Their recommendations, among others, address the question of inappropriate prediction of patient outcomes based on biomarkers.

Salivaomics has been extensively studied during the last two to three decades [4, 5]. Normal saliva is a complex mixture of secretions of digestive enzymes, mucin, hormones, electrolytes etc., from the three pairs of major and several minor salivary glands and includes non-salivary components like de-squamated cells, bacteria, bacterial byproducts, other debris and blood components from oral disease. It is also a rich secretion of antimicrobials and immuno-modulatory proteins. Salivary proteome has already been analysed. The salivary glands process saliva through simple filtration, passive diffusion and active transport systems from their rich vasculature. Like other body fluids, saliva often reflects the tissue fluid levels of therapeutic, hormonal and immune molecules as well as biomarkers of systemic and oral disease that are derived locally or from remote areas. Only a rigorous study can evaluate their clinical application.

In a well-designed study in 50 healthy young adult volunteers from university campuses, Williamson et al. [6] evaluated the levels of an array of 27 biomarkers *simultaneously in*

✉ Sushila Sripad
drssripad@gmail.com

[1] Rabindranath Tagore International Institute of Cardiac Sciences, Mukundapur, Kolkata, India

[2] Former Head, Department of Cardiovascular and Thoracic Surgery, Medical College, Calcutta, 88 College Street, Kolkata, India

the plasma and saliva. They also evaluated the methods of collection of saliva. They concluded that saliva collected by the passive drool method is the better method and that there was a significant correlation of only 3/27 biomarkers, namely IL-6, IFN-gamma and MIP-1 beta, in plasma and saliva. However, they cautioned that since the oral cavity represents a 'niche' environment, cytokine levels in saliva cannot be used as surrogate markers for systemic immune response.

Rathnayake et al. [7], in a *Swedish random population-based study* of 441 adults aged 21–89 years found detectable *salivary* levels of IL-1b, IL-6, IL-8, MMP-8, TIMP-1 and lysozyme in more than 99% of the samples suggesting an inflammatory response of local or systemic origin.

Salivary biomarkers have been most useful in the study of cortisol levels in Cushing's disease, biomarkers in Sjogren's disease, periodontal disease and oral cancer. IL-8 was consistently elevated in oral and head and neck squamous cell carcinoma and could be used as a potential biomarker for *epidemiological* screening. Salivary BRCA1 and BRCA2 and HER2 mutations have been identified in breast cancer, but clinical decision-making based on these requires rigorous clinical evaluation and validation. Lack of such rigorous study and validation, at that time, was the cause for the US Supreme Court decision 1993 curbing some of those practices. Currently, they have been validated and have been used for screening of high-risk individuals for genetic counselling and/or prophylactic treatments.

Atherosclerosis is now established, to be the result of chronic low-level inflammation in the vascular endothelium. Heinisch et al. [8], in a well-controlled and well-designed study, measured serial changes in *plasma* cytokines in controls and patients with CAD presenting with stable angina (SA) and those with acute coronary syndrome (ACS). They found (i) very high levels of TNFα, IL-6, IFNγ and C-reactive protein (CRP) in ACS, less so in SA; (ii) at admission, IL 6 was elevated in 50% of ACS vs only 5% of patients of SA; (iii) 35% of ACS showed elevation of TNFα; (iv) with treatment, IL-6 declined until it was undetectable while TNFα progressively increased and was detectable at all times; and (v) IFNγ was detectable *only* in ACS at levels of 304.1 ± 254.4 pg/ml. They also found a significant positive correlation between CRP and IL-6 ($r = 0.4$; $p < 0.01$). Since CRP is done routinely in patients of SA presenting for admission, the role of the expensive *plasma* IL-6 as a biomarker may be debatable, so would be the case with salivary IL6.

There is a vast volume of literature on *normal* responses to aerobic and resistance exercises in different age groups and in post stress situations of all categories not necessarily due to CAD. Major surgery itself gives rise to stress response and biomolecules [9, 10]. Saitoh et al. [11] demonstrated that high macrophage colony-stimulating factor (MCSF) values (> 950 pg/ml) *predicted* cardiac events during a 14-month follow-up in a mixed population of 97 patients with stable and 45 patients with unstable angina. IL 6 *plasma* markers were not conclusive in predicting future events. Salivary IL6 is unlikely to be better. They suggested that larger studies may determine the *prognostic* value of MCSF and its importance vis-a-vis that of C-reactive protein, a reliable, low cost and extensively studied inflammatory marker.

Later in this issue, Vasaghi-Gharamaleki et al. [1], from a cardiac rehabilitation unit from Teheran, attempted to study the changes in the levels of the salivary biomarker IL-6 in a select group of 30 male patients in the 45- to 75-year age group who underwent *single-vessel* CABG 4–6 weeks earlier, to *predict future events* based on the impact of 8 weeks of exercise, on salivary biomarker IL6 and anthropometric measurements. This select group had many exclusions without any information on objective data and would be considered a low-risk group.

In the background of the vast literature on the subject of salivaomics, IL-6, CAD, stress response, cardiac rehabilitation exercises etc. as briefly outlined above, the study suffers from many infirmities in design that would be expected to affect the conclusions. The authors also mention about 'visceral fat' but there is no information on how they came to that conclusion because clinical details of evaluation for visceral fat have not been given. There is no information on the surgical aspects, viz. nature, location and extent of CAD, anaesthesia, on-pump/off-pump, duration of surgery, duration of post operation, ventilation and intensive care, nature/doses/duration of medicines and diet that the patients were receiving and break-up of age groups, all of which are highly relevant for interpretation of the results. For instance, levels of serum/plasma biomarkers in response to exercise vary with age even in normal subjects.

The timing of the exercise intervention, interpretation of the anthropometric data and duration of study could have been better. Stress response has at least three phases of variable duration. The anthropometric data presented suggest that the patients were still gaining weight and were in the anabolic phase, and the recovery could last 6–12 months or even longer [9].

Though considerable advances in point-of-care (POC) salivary diagnostic technology have been made [5], salivaomics in systemic disease such as CAD is still in the research stage and rigorous study is required. For instance, serial estimations simultaneously in serum/plasma and saliva (collected by the drool method), over a period of at least several months of continued supervised cardiac rehabilitation in a moderate-to high-risk CAD group of matched patients post CABG, using a broader panel of biomarkers, e.g. MCSF, TNFα, IL-6, IFNγ and hsCRP, may have improved the study.

Besides, IL-6 encoded by the IL6 gene in the humans has both pro-inflammatory and anti-inflammatory properties [12]. Only a few cells (hepatocyte and leucocyte membranes) express IL6 receptor and respond to IL6 as an anti-inflammatory

myokine, in the *classic signalling mode*. IL 6 as a pro-inflammatory cytokine (e.g. in endothelial dysfunction) correlates well with CRP-an extensively studied and validated low-cost biomarker. Petrak et al. [13] found that IL 6 is the most important cytokine released locally from muscles during exercise, peaks around 2 1/2 h of running and has a half-life of 1–2 h. They suggest that these changes may be due to native adrenaline secretion but may also be related to the duration of exercise, type of exercises etc. Therefore, the *timing* of sampling of any body fluid, for an assay to study the true long-term impact of this biomarker in cardiac rehabilitation, needs to be carefully chosen.

Current salivary diagnostics, specially nano-chip multiplex technology, offering a reproducible detection of an array of specific biomarkers in CAD in a properly designed and analysed study may be an interesting prospect. Once *validated*, such "lab-on-chip" [5] POC technology may soon be available for CAD as well as for many other systemic diseases and be used as bedside diagnostics for screening/predicting outcomes or in epidemiological studies.

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