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Detection of Oxidative Stress in Buccal Cells using iSWAB Tubes

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Abstract

Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis in favor of oxidants leading to the oxidation of DNA, lipids, and proteins. In this experiment, we investigated the oxidative profile of iSWAB-Mawi protein tubes by examining their ability to detect the protein oxidation biomarkers: nitrotyrosine and S-nitrosocysteine in buccal cells in a sample of 40 participants, equally divided between Lebanese University and Benta Pharma Industries. Protein concentrations were determined by Bradford assay in order to then be analyzed for the presence of nitrotyrosine and S-nitrosocysteine by western blot. Results showed the presence of S-nitrosocysteine and nitrotyrosine in buccal cells reflecting the ability of iSWAB-Mawi protein tubes to detect those protein oxidation biomarkers.

Keywords: Oxidative stress; iSWAB-Mawi tubes; Buccal cells; S-nitrosocysteine; Nitrotyrosine

Introduction

Oxidative stress is defined as the disturbed balance between reactive species and antioxidants in favor of the pro-oxidants. When this imbalance takes place, oxidation of proteins, lipids, and DNA arises resulting in aging and several diseases including: cancer, cardiovascular diseases, and neurodegenerative diseases [1]. Reactive species are divided into free radicals and non-radicals and can be classified into 3 categories which are: reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) [2].

Nitric oxide (NO·) is one of the main free radicals that have gained great attention recently. It is synthesized from L-arginine by means of nitric oxide synthase (NOS) converting L-arginine to L-citrulline [3,4]. Once produced, $NO^{\cdot}$ could act either directly or indirectly. Direct action is either via: 1) soluble guanylyl cyclase (sGC) activation which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP); a crucial intracellular messenger, 2) via the action of NO on the thiol group which results in S-nitrosothiols (SNO) formation. However, the indirect action occurs via production of other reactive oxygen/nitrogen species formed by combination of two independent radical generation pathways such as the reaction between $O_2^{\cdot -}$ and NO leading to the formation of peroxynitrite ($ONOO^-$)which is a potent oxidant [4,5].

Although NO per se results in physiological and pathophysiological consequences such as smooth muscle relaxation and neurodegenerative diseases, it was shown that many NO effects appear through secondary byproducts formation. Crucial consequences of NO appeared to result from peroxynitrite and S-nitrosothiols [6,7].

Peroxynitrite

Peroxynitrite ($ONOO^-$) is a short-lived strong oxidant that results from the reaction between superoxide and nitric oxide [8]. It plays a dual role; on one hand, nitric oxide scavenges superoxide which has damaging effect. On the other hand, the reaction reduces the cardioprotective molecule, $NO^{\cdot}$, and produces a detrimental molecule which is peroxynitrite. Thus, peroxynitrite formation is implicated in both beneficial effects such as its vaso relaxant effect in vascular smooth muscle cells, and deleterious effects such as myocardial contractile dysfunction [9-11].

The most predominant reaction of $ONOO^-$ is with tyrosine, known as nitration which is an irreversible oxidative posttranslational modification consisting of the binding of a nitro group ($-NO_2$) to the aromatic ring of tyrosine residue [12]. Due to peroxynitrite's high affinity for tyrosine residues in proteins, 3-nitrotyrosine has been used as a footprint of peroxynitrite-mediated nitrative stress of proteins that can be detected and measured *in vivo* and is correlated with several diseases [8].

Nitrotyrosine plays pathophysiological as well as physiological role in the body such as cell signaling via tyrosine kinase pathway and implication in heart dysfunction [13,14].

S-nitrosocysteine

S-nitrosylation is defined as a reversible posttranslational modification of a protein in which nitric oxide reacts with the thiol group of cysteine residues to form S-nitrosocysteine that is considered one of the NO-mediated nitrosative stressbiomarkers [15,16].

The beneficial effects of S-nitrosylation have gained great importance in the cardiovascular system where it was shown that *S*-nitrosylation of ryanodine receptor 2 (RYR2) in the heart amends heart failure by reversing calcium leakage [16].

Although S-nitrosylation seems to have an important role in signal transduction, its dysregulation leads to many pathophysiological effects that results in a broad spectrum of human diseases. Studies showed that S-nitrosylation of anti-apoptotic proteins is a major aspect of tumor biology and cancerogenesis [17].

Because buccal cells provide an accessible pool of epithelial cells, they are considered as a powerful diagnostic tool for evaluating oxidative damage [18]. They can also mirror systemic health status, so people at risk for several diseases can be clarified by the identification of biomarkers that assess the outcomes of ROS in the oral cavity. Such

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biomarkers can also be useful in clinical trials to measure the toxicity of drugs that influence redox homeostasis [19]. Using buccal cells to isolate proteins gain is superior over blood collection mainly because it is non-invasive and enables self-collection (no expertise is needed), which makes it suitable for all population segments. Buccal cells collection also represents better option over saliva collection although both are non-invasive. However, it seems difficult for people having xerostomia (dry mouth) to provide saliva [20].

Different methods for protein collection from buccal cells can be used like cytobrush, mouthwash, and swabs [21].

A new collection kit, the iSWAB-Protein collection tubes, have immerged to allow for collection and stabilization of proteins at the point of collection. The unique design of Mawi-iSWAB protein tubes enables mechanistic release of cells into a proprietary lysis and stabilization buffer, resulting in the recovery of nearly all cells from single or several swabs while significantly minimizing bacterial contamination.

iSWAB-Mawi tubes have several advantages. Most importantly, Mawi tubes allow the preservation of proteins for up to 6 months and are stable at room temperature which reduces sample storage and transport costs by eliminating cold chain requirements. Moreover, they are simple, easy, and fast for sample collection because self-collection can be achieved in less than 5 minutes. They are also characterized by the Swab-free sample transport which decreases sample processing time. Mawi tubes contain alcohol-free buffer which results in better yields of proteins, prevents interference with enzyme immunoassays-based detection methods, and allows for high sensitivity testing [22].

In an attempt to evaluate the extent of protein oxidation and due to the importance of protein oxidation biomarkers in assessing the profile of people at risk of having a disease, Mawi-iSWAB protein tubes were used for further testing and detection of protein oxidation biomarkers: Nitrotyrosine and S-nitrosylation.

Material and Methods

Study population

Informed consent forms for the collection of human buccal cells were obtained from twenty instructors from the Faculty of sciences and Ecole Doctoral of the Lebanese University, Hadath campus, and twenty employees from Benta Pharma Industries, Dbayeh. Participants also provided self-reported data stating the gender, age, smoking, alcohol consumption and health conditions such as hypertension, diabetes, atherosclerosis, asthma, etc.

Buccal cells collection

Prior to the collection of each sample, participants were asked to rinse their mouth two times with water, swabbing was performed thirty minutes after the rinse by rotating a cotton swab twenty times on the inner of the cheek and repeated for four times. Swabs were released into a 1 mL iSWAB™ Protein (iSWAB-P-1200) tube for sample collection.

Sample processing and protein extraction

Samples were kept at room temperature before protein extraction and protein lysates were processed four hours post collection. Samples were vortexed briefly and centrifuged at 1000x g for one minute at 25°C. Supernatants were then removed for protein concentration evaluation by Bradford assay (protocol of Sigma Aldrich, Catalog Number B6916) and read by spectrophotometer at 595 nm. Standard curve was demonstrated starting from BSA=2 mg/mL. Each tube was assayed in duplicates.

Laemmli reducing buffer (BioRAD) containing Tris-HCl, β-mercaptoethanol, SDS, glycerol, and bromophenol blue was then added to the samples. Samples were boiled for three minutes. Western blot was then performed as described below.

Control cells processing

Control cells were collected and processed without using iSWAB-Mawi tubes to ensure that it is not the buffer per se in those tubes that is responsible for generation of reactive species and subsequently protein oxidation. Control cells were collected and processed [23] where 2 washes were obtained by brushing the inside of the oral cavity by a sterile toothbrush. Buccal cells were then pelleted by centrifugation. Bradford assay (protocol of Sigma Aldrich, Catalog Number B6916) was performed. After Bradford, pellets obtained were sonicated. Western blot was then applied as described below. Negative control was Mawi's buffer alone to ensure that the biomarkers are not present in the buffer itself. Molecular weights of the bands obtained from the samples were compared to a broad range MW ladder (BioRAD, 161-0317).

Western blot

15 µg/sample were loaded in each well of a 12% polyacrylamide gels that were run at 80 volts for two hours. Gels were then transferred to a nitrocellulose membrane (BioRad). Membranes were then stained with ponceau S. red for twenty minutes and visualized by versa Doc imaging system (Bio-RAD).

Membranes were then blocked with 5% BSA (affymetrix) and incubated overnight at 4°C.

Membranes were washed three times with PBST for five minutes then incubated with primary antibodies for one hour on a rocker at room temperature. Primary antibodies were: mouse monoclonal anti-nitrotyrosine (Abcam ab7048), mouse monoclonal anti-S-nitrosocysteine (Abcam ab94930). Membranes were then washed three times with PBST for five minutes and incubated with secondary antibody for one hour at room temperature. Secondary antibody was: HRP-conjugated rabbit anti-mouse polyclonal antibody (Abcam ab97046).

ECL (BioRAD 170-5060) or TMB (Sigma Aldrich) was added to the membranes for visualization using Versa Doc imaging system.

Results

In this study, Bradford assay was used to determine the total concentration of the proteins in the sample. Proteins obtained from the academic group provided a mean concentration of 0.86 mg/mL. Concentrations of the LU samples are represented in Table 1. Proteins obtained from the industrial group yielded a mean concentration of 0.77 mg/mL. Table 2 represents the concentration of each sample obtained from BPI participants. Concentration of control cells obtained is 0.9529 mg/mL × dilution factor (2)=1.9058 mg/mL.

The presence of the protein oxidation biomarkers S-nitrosocysteine and nitrotyrosine was detected by western blots. Each sample was tested for the presence of both S-nitrosocysteine and nitrotyrosine in two different membranes.

In Figures 1-6, bands of molecular weights ranging between 30 KDa and 60 KDa revealed apparent presence of nitrotyrosine in buccal cells.

Sample name	Absorbance of replicate 1 (595 nm)	Concentration of replicate 1 (mg/mL)	Absorbance of replicate 2 (595 nm)	Concentration of replicate 2 (mg/mL)
1	0.6488	0.7691	0.6479	0.7681
2	0.6567	0.7784	0.6642	0.7874
3	0.6664	0.79	0.6589	0.7811
4	0.6749	0.8	0.6659	0.7894
5	0.645	0.7646	0.6625	0.7854
6	0.6478	0.7679	0.6495	0.7699
7	0.6369	0.7551	0.64	0.7593
8	0.6353	0.7532	0.6526	0.7737
9	0.6429	0.7621	0.6516	0.7724
10	0.6245	0.74	0.6345	0.7522
11	0.6629	0.7858	0.6807	0.8
12	0.6449	0.7645	0.644	0.7634
13	0.6658	0.7894	0.675	0.8
14	0.6631	0.786	0.6714	0.7959
15	0.6574	0.7793	0.6587	0.78
16	0.6624	0.7852	0.6676	0.7915
17	0.68	0.8062	0.6717	0.7963
18	0.6533	0.7744	0.6551	0.7765
19	0.66	0.79	0.66	0.788
20	0.6893	0.8172	0.6833	0.81

Table 1: Evaluation of of protein concentrations obtained from 20 participants from the faculty of sciences and Ecole doctorale of the Lebanese University using Bradford assay.

Sample name	Absorbance of replicate 1 (595 nm)	Concentration of replicate 1 (mg/mL)	Absorbance of replicate 2 (595 nm)	Concentration of replicate 2 (mg/mL)
1	0.4352	0.8721	0.4285	0.8588
2	0.4564	0.9147	0.4463	0.8944
3	0.443	0.8879	0.4413	0.8844
4	0.4385	0.8787	0.4468	0.8954
5	0.4663	0.9345	0.4533	0.9085
6	0.443	0.8878	0.4261	0.854
7	0.64	0.8492	0.6665	0.8844
8	0.6418	0.8516	0.6169	0.8185
9	0.6498	0.8622	0.6412	0.8508
10	0.6525	0.8657	0.6761	0.8971
11	0.6491	0.8612	0.6332	0.8401
12	0.5763	0.7647	0.6234	0.8272
13	0.6467	0.8581	0.6504	0.863
14	0.6859	0.9101	0.6961	0.9237
15	0.679	0.901	0.7011	0.9303
16	0.6278	0.8329	0.6349	0.8424
17	0.6364	0.8443	0.6243	0.8284
18	0.7069	0.938	0.7022	0.9317
19	0.6504	0.863	0.6477	0.8593
20	0.6312	0.8375	0.6411	0.8507

Table 2: Evaluation of the protein concentrations obtained from 20 participants from BPI, Dbayeh using Bradford assay.

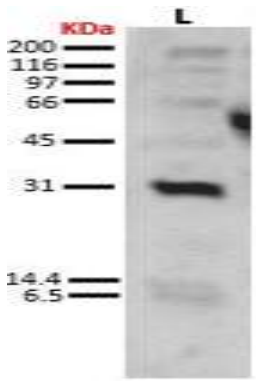

Figure 1: Detection of nitrotyrosine in buccal cells of 8 LU samples by western blot. L: ladder; C: control cells; Lanes 1 till 8: proteins obtained from LU samples. 15 µg/Lane.

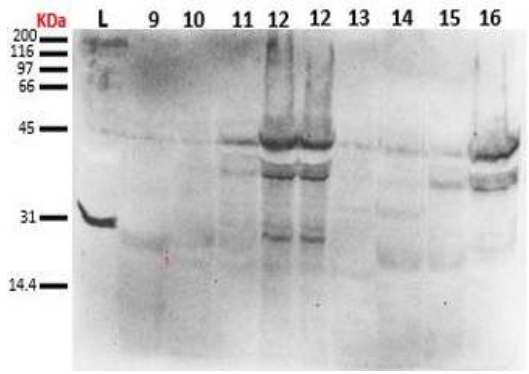

Figure 2: Detection of nitrotyrosine in buccal cells of 8 LU samples by western blot. L: ladder; C: control cells; Lanes 9 till 16: proteins obtained from LU samples. 15 µg/Lane.

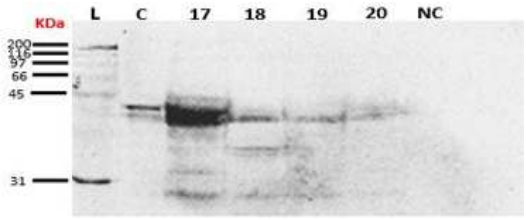

Figure 3: Detection of nitrotyrosine in buccal cells of 4 LU samples by western blot. L: ladder; C: control cells; Lanes 17 till 20: proteins obtained from LU samples; NC: negative control. 15 µg was loaded in each lane.

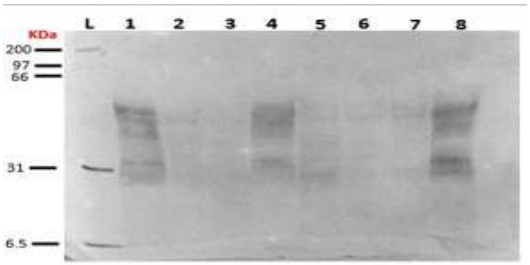

Figure 4: Detection of nitrotyrosine in buccal cells of 8 BPI samples by western blot. L: ladder; Lanes 1 till 8: proteins obtained from BPI samples. 15 µg/Lane.

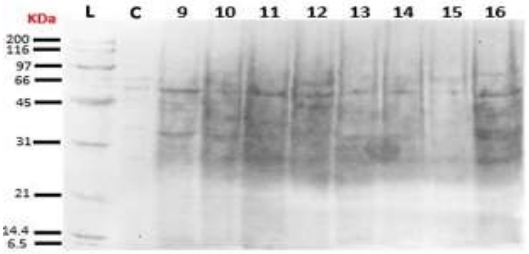

Figure 5: Detection of nitrotyrosine in buccal cells of 8 BPI samples by western blot. L: ladder; Lanes 9 till 16: proteins obtained from BPI samples. 15 µg/Lane.

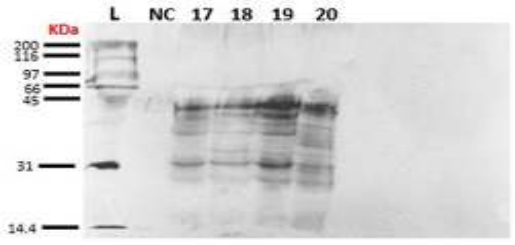

Figure 6: Detection of nitrotyrosine in buccal cells of 4 BPI samples by western blot.L: ladder; C: control cells; Lanes 17 till 20: proteins obtained from LU samples; NC: negative control. 15 µg was loaded in each lane.

In Figures 1-3, samples 5-8 showed bands of higher intensity than the other 4 LU samples.

Also, bands obtained from BPI samples are of higher intensity than that of LU.

In Figures 7-12, bands of molecular weights between 30 KDa and 50 KDa appeared in all samples which revealed the presence of S-nitrosylated proteins in buccal cells.

As shown in the figures, bands of the control cells were obtained in all membranes and the bands in the negative control lanes were absent.

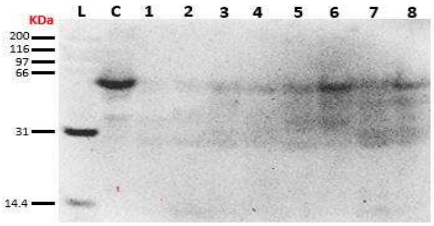

Figure 7: Detection of S-nitrosocysteine in buccal cells of 8 LU samples by western blot.L: ladder;C:control cells;Lanes 1 till 8:proteins obtained from LU samples. 15 µg/Lane.

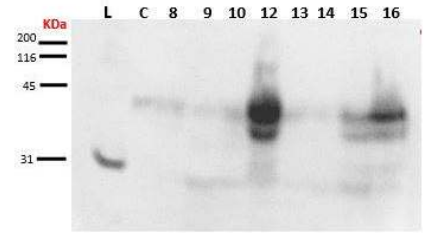

Figure 8: Detection of S-nitrosocysteine in buccal cells of 8 LU samples by western blot.L: ladder;C:control cells;Lanes 1 till 8:proteins obtained from LU samples 15 µg/Lane.

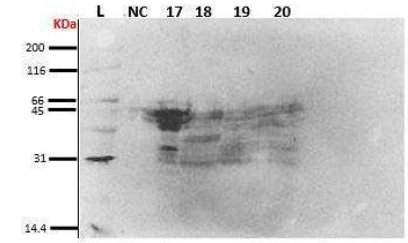

Figure 9: Detection of S-nitrocysteine in buccal cells of 4 LU samples by western blot.L: ladder; C: control cells; Lanes 17 till 20: proteins obtained from LU samples; NC: negative control. 15 µg was loaded in each lane.

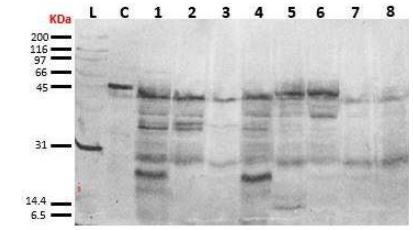

Figure 10: Detection of S-nitrosocysteine in buccal cells of 8 BPI samples by western blot. L: ladder; C: control cells; Lanes 1 till 8: proteins obtained from BPI samples 15 µg/Lane.

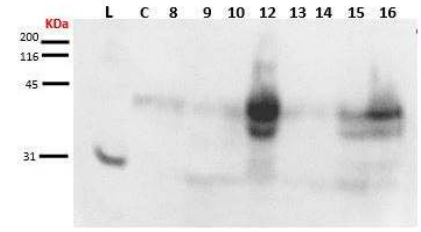

Figure 11: Detection of S-nitrosocysteine in buccal cells of 8 BPI samples by western blot. L: ladder; Lanes 9 till 16: proteins obtained from BPI samples 15 µg/Lane.

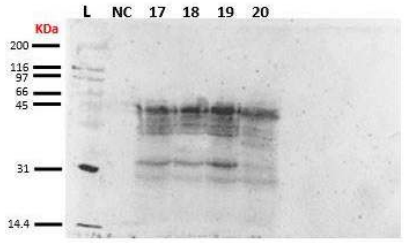

Figure 12: Detection of S-nitrocysteine in buccal cells of 4 BPI samples by western blot.L: ladder; C: control cells; Lanes 17 till 20: proteins obtained from LU samples; NC: negative control. 15 µg was loaded in each lane.

Discussion

The 2 groups were almost of the same ages both being young adults of the same ethnicity i.e. Caucasians. Also, there was no significant difference based on weights, smoking profile, antioxidant consumption, and regular exercising. However, the only between-groups variables were the gender and alcohol consumption profile where the academic population showed less alcohol consumption than the BPI population. The number of female participants dominates the number of males in the industry. However, in Lebanese University, the number of females was lower than the number of males.

It was previously shown that there is an evident association between gender and oxidative stress where males are more prone to oxidative stress than females [24]. This may be linked to the protective effects of estrogen by acting itself as an antioxidant due to the presence of phenolic hydroxyl group. It was also shown that the gene expression of SOD and GPx is estrogen-dependent. Moreover, estrogen lowers the levels of NADPH oxidases which explains the fact that females have lower levels of NADPH oxidases and thus reduced superoxide production [25].

The variation of the bands intensities was correlated to the lifestyle and health condition of the participant.

By correlating samples 5-8 with their lifestyles, we found out that participant 5 was a heavy smoker of 40 cigarettes per day, participant 6 was diagnosed with hypercholesterolemia, and participant 8 was a hookah smoker and alcohol consumer.

The high intensity bands obtained in BPI samples can be associated with alcohol consumption, the most pronounced variable between the 2 groups.

Oxidative metabolism of alcohol is controlled by 3 enzymatic systems that metabolizes alcohol into acetaldehyde and include: alcohol dehydrogenase (ADH) in the cytosol which requires NAD^+ and accounts for 90% of the consumed alcohol, CYP 450 2E1-dependent microsomal ethanol oxidizing system (MEOS) in the microsome, and catalase in peroxisome [26]. The high amounts of NAD+ and NADPH produced are responsible for the increase in the generation of reactive species.

The presence of the bands of the control cells in all membranes indicated that the buffer of the iSWAB-Mawi protein tubes was not related to the oxidation of proteins. Besides, the absence of bands in the negative control lanes ensured that the buffer alone didn't contain the protein oxidation biomarkers.

Thus, it can be inferred that Mawi-iSWAB protein tubes are able to detect those biomarkers and that the buffer doesn't scavenge them.

Conclusion

In conclusion, iSWAB-Mawi protein tubes are able to detect the protein oxidation biomarkers: nitrotyrosine and S-nitrosocysteine. Those findings may be of great help for researchers in terms of storage and transport during protein oxidation researches.

The gap in the literature that served as a motivation for this research has been partially filled. However, future research implications stem out of these findings. One of them is to find whether iSWAB-Mawi protein tubes can not only detect nitrotyrosine and S-nitrosocysteine, but also preserve those biomarkers. This could be accomplished by performing stability studies to examine the effects of Mawi's buffer, or even the influence of the environmental factors such as humidity and light on the ability of Mawi's protein tubes to preserve the protein oxidation biomarkers: nitrotyrosine and S-nitrosocysteine.

References

1. Biswas SK (2016) Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid Med Cell Longev 2016: 17-19.
2. Asmat U, Abad K, Ismail K (2016) Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J 24: 547-553.
3. Kasiappan R, Safe SH (2016) ROS-inducing agents for cancer chemotherapy. ROS 1: 22-37.
4. Rahman T, Hosen I, Islam MMT, Shekhar HU (2012) Oxidative stress and human health. Adv Biosci Biotechnol 3: 997-1019.
5. Habib S, Ali A (2011) Biochemistry of nitric oxide. Indian J Clin Biochem 26: 3-17.
6. Antosova M, Plevkova J, Strapkova A, Buday T (2012) Nitric oxide-important messenger in human body. Open J Mol Integr Physiol 2: 98-106.
7. Jerca L, Jerca O, Mancaş G, Constantinescu I, Lupuşoru R (2002) Mechanism of action and biochemical effects of nitric oxide. J Prev Med 10: 35-45.
8. Ahmad R, Ahsan H (2011) Contribution of peroxynitrite, a reactive nitrogen species, in the pathogenesis of autoimmunity. Intech 8: 141-156.
9. Bassil M, Li Y, Anand-srivastava MB (2008) Peroxynitrite inhibits the expression of G i alpha protein and adenylyl cyclase signaling in vascular smooth muscle cells. J Physiol Hear Circ Physiol 7: 775-785.
10. Ronson RS, Nakamura M, Vinten-johansen J (1999) The cardiovascular effects and implications of peroxynitrite. Cardiovasc Res 44: 47-59.
11. Vinten-johansen J (2000) Physiological effects of peroxynitrite. Circ Res 87: 170-173.
12. Corpas FJ, Del Río LA, Barroso JB (2008) Post-translational modifications mediated by reactive nitrogen species: Nitrosative stress responses or components of signal transduction pathways? Plant Signal Behav 3: 301-303.
13. Kamisaki Y, Wada K, Bian Ka, Balabanli B, Davis K, et al. (1998) An activity in rat tissues that modifies nitrotyrosine-containing proteins. Proc Natl Acad Sci USA 95: 11584-11589.
14. Gould N, Doulias PT, Tenopoulou M, Raju K, Ischiropoulos H (2013) Regulation of protein function and signaling by reversible cysteine S-nitrosylation. J Biol Chem 288: 26473-26479.
15. Salvemini D, Doyle TM, Cuzzocrea S (2006) Superoxide, peroxynitrite and oxidative/nitrative stress in inflammation. Biochem Soc Trans 34: 965-970.
16. Gonzalez DR, Beigi F, Treuer AV, Hare JM (2007) Deficient ryanodine receptor S-nitrosylation increases sarcoplasmic reticulum calcium leak and arrhythmogenesis in cardiomyocytes. Proc Natl Acad Sci 104: 20612-20617.
17. Iyer AK, Azad N, Wang L, Rojanasakul Y (2011) Role of S-nitrosylation in apoptosis resistance and carcinogenesis. Nitric Oxide 19: 146-151.
18. Borthakur G, Butryee C, Stacewicz-sapuntzakis M, Bowen PE (2008) Exfoliated buccal mucosa cells as a source of DNA to study oxidative stress. Cancer Epidemiol Biomarkers Prev 17: 212-220.
19. Grek CL, Reyes L, Townsend DM, Tew KD (2014) S-glutathionylation of

buccal cell proteins as biomarkers of exposure to hydrogen peroxide. BBA Clin 2: 31-39.

20. Gibbins HL, Proctor GB, Yakubov GE, Wilson S, Carpenter GH (2014) Concentration of salivary protective proteins within the bound oral mucosal pellicle. Oral Disno 20: 707-713.
21. Livy A, Lye S, Jagdish CK, Hanis N, Sharmila V, et al. (2012) Evaluation of quality of DNA extracted from buccal swabs for microarray based genotyping 27: 28-33.
22. http://www.mawidna.com/products/iswab-protein-collection-kit
23. Michalczyk A, Varigos G, Smith L, Ackland ML (2004) Fresh and cultured buccal cells as a source of mRNA and protein for molecular analysis. Biotechniques 37: 262-269.
24. Kander MC, Cui Y, Liu Z (2017) Gender difference in oxidative stress : a new look at the mechanisms for cardiovascular diseases. J Cell Mol Med 21: 1024-1032.
25. Cui H, Kong Y, Zhang H (2012) Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 2012: 646354.
26. Dekker J, Elamin EE, Masclee AA, Dekker J, Jonkers DM (2013) Ethanol metabolism and its effects on the intestinal epithelial barrier Ethanol metabolism and its effects on the intestinal epithelial barrier. Nutr Rev 71: 483-499.