

Effect of Locally Delivered Antioxidants as An Adjunct to Non-surgical Periodontal Therapy on GCF Level of Oxidative Stress Marker; Protein Carbonyl

Proposal submitted in partial fulfillment of the requirements for
Master Degree in Oral medicine, Periodontology and Oral diagnosis

Maie Sami Tawfik Sharaf

B.D.S 2010

Faculty of dentistry-Ain Shams University

Supervised by:

Prof. Dr. Khaled Atef Abdel-Ghaffar

**Professor of Oral Medicine, Periodontology, Oral Diagnosis and Radiology
Minister of Higher Education and Scientific research**

Dr. Fatma Hamed Mohammed El-Demerdash

**Lecturer of Oral Medicine, Periodontology, Oral Diagnosis and Radiology
Faculty of Dentistry, Ain Shams University**

**Faculty of Dentistry
Ain Shams University
2017**

LIST OF CONTENTS

<i>LIST OF CONTENTS</i>	<i>I</i>
<i>LIST OF FIGURES</i>	<i>II</i>
<i>LIST OF TABLES</i>	<i>IXII</i>
<i>INTRODUCTION AND REVIEW OF LITERATURE</i>	<i>1</i>
<i>AIM OF THE STUDY</i>	<i>38</i>
<i>SUBJECTS AND METHODS</i>	<i>39</i>
<i>CASE PRESENTATION</i>	<i>55</i>
<i>RESULTS</i>	<i>64</i>
<i>DISCUSSION</i>	<i>64</i>
<i>CONCLUSION</i>	<i>84</i>
<i>REFERENCES</i>	<i>85</i>
<i>ARABIC SUMMARY</i>	<i>1</i>

LIST OF FIGURES

Figure 1	<i>A digramatic representation shows oxidative stresses generation and their effect on different cell structures adapted from Kelly F. et al., 2003.</i>	3
Figure 2	<i>A schematic representation of the NADPH-oxidase shunt, demonstrating the use of glucose-6-phosphate (G6P) and NADPH to effect the single electron reduction of oxygen to superoxide adapted from Chapple IL et al., 2007.</i>	5
Figure 3	<i>A schematic representation of the role of bacteria and their products in receptor mediated neutrophil ROS production adapted from Chapple IL et al., 2007</i>	7
Figure 4	<i>A diagrammatic representation shows free radical chain reaction and the effect of chain breaking antioxidants.</i>	11
Figure 5	<i>A diagrammatic representation of functional regeneration of oxidative stresses by host defense cells within subgingival plaque biofilm adapted from Milward MR, Chapple ILC, 2013.</i>	16
Figure 6	<i>A diagrammatic representation of effects of a diet high in refined sugar, carbohydrate and saturated fat on neutrophil function adapted from Milward MR, Chapple ILC, 2013.</i>	18

Figure 7	<i>A schematic representation of the effects of ROS proteins and amino acids adapted from Dean et al., 1997.</i>	22
Figure 8	<i>A diagrammatic representation of Structure of solid lipid nanoparticles and solid lipid microparticles stabilized by surfactant layer adapted from adapted from Elwira lason et al., 2011.</i>	37
Figure 9	<i>Showing pure lycopene of 96 % HPLC.</i>	43
Figure 10	<i>Showing the sustained release form of lycopene.</i>	44
Figure 11	<i>Showing plastic bite-block, plastic aiming ring, metal indicator arm and silicine bite-block.</i>	47
Figure 12	<i>Showing Soredex Classic Digora® Optime</i>	48
Figure 13	<i>Showing Digora Optime Imaging-plate</i>	48
Figure 14	<i>Showing digital radiograph taken by parallel technique</i>	49
Figure 15	<i>Woodpecker UDS-K LED ultrasonic scaler</i>	50
Figure	<i>Periopaper GCF collection strips</i>	51

16		
Figure 17	<i>A diagram summarizing steps of ELISA test.</i>	53
Figure 18	<i>Baseline clinical photograph of a periodontal pocket with probing depth of 8 mm mesio-buccal to upper left first molar.</i>	56
Figure 19	<i>Baseline digital radiograph showing interdental infrabony defect mesial to upper left first molar measuring 3.8 mm.</i>	57
Figure 20	<i>A photograph showing collection of GCF using PerioPaper® GCF Collection Strips thirty minutes after SRP.</i>	57
Figure 21	<i>Local delivery of sustained release form of lycopene to the test site followed by another GCF sample for measuring rate of drug release.</i>	58
Figure 22	<i>Six months postoperative clinical photograph of the periodontal pocket with probing depth measuring 3 mm.</i>	58
Figure 23	<i>Six months postoperative digital radiograph showing interdental infrabony defect measuring 3.3 mm.</i>	Error! Bookmark not defined.
Figure 24	<i>Baseline clinical photograph of a periodontal pocket with probing depth of 6.5 mm mesio-buccal to upper left second molar.</i>	Error! Bookmark not defined.

Figure 25	<i>Baseline digital radiograph showing interdental infrabony defect mesial to upper right first molar measuring 3.2 mm.</i>	Error! Bookmark not defined.
Figure 26	<i>A photograph showing collection of GCF using PerioPaper® GCF Collection Strips thirty minutes after SRP</i>	Error! Bookmark not defined.
Figure 27	<i>Six months postoperative clinical photograph of the periodontal pocket with probing depth measuring 2.5 mm.</i>	Error! Bookmark not defined.
Figure 28	<i>Six months postoperative digital radiograph showing crestal bone loss with interdental infrabony defect measuring 2.4 mm.</i>	Error! Bookmark not defined.
Figure 29	<i>Clinical photograph of a gingival sulcus with probing depth of 2.5 mm mesio-buccal to upper left first premolar.</i>	Error! Bookmark not defined.
Figure 30	<i>A photograph showing collection of GCF using PerioPaper® GCF Collection Strips.</i>	Error! Bookmark not

		<i>defined.</i>
Figure 31	<i>Baseline radiograph showing zero defect mesial to upper right first premolar.</i>	<i>Error! Bookmark not defined.</i>
Figure 32	<i>Bar chart showing groups' mean values of PC at different follow-up periods.</i>	<i>Error! Bookmark not defined.</i>
Figure 33	<i>Linear chart showing the mean PC concentrations at different follow-up periods.</i>	<i>Error! Bookmark not defined.</i>
Figure 34	<i>Bar chart showing groups' mean values of PI at different intervals.</i>	<i>Error! Bookmark not defined.</i>
Figure 35	<i>Linear chart showing the mean values of PI at different follow-up periods.</i>	<i>Error! Bookmark not defined.</i>
Figure 36	<i>Bar chart showing groups' mean values of MGI at different intervals.</i>	<i>Error! Bookmark</i>

		<i>not defined.</i>
Figure 37	<i>Linear chart showing the mean values of MGI at different follow-up periods.</i>	Error! Bookmark not defined.
Figure 38	<i>Bar chart showing groups' mean values of PD at different intervals.</i>	Error! Bookmark not defined.
Figure 39	<i>Linear chart showing the mean values of PD at different follow-up periods.</i>	Error! Bookmark not defined.
Figure 40	<i>Bar chart showing groups' mean values of CAL at different intervals.</i>	Error! Bookmark not defined.
Figure 41	<i>Linear chart showing the mean values of CAL at different follow-up periods.</i>	Error! Bookmark not defined.
Figure	<i>Bar chart showing groups' mean values of intrabony defect measure at different intervals.</i>	Error!

42		<i>Bookmark not defined.</i>
<i>Figure 43</i>	<i>Linear chart showing the mean values of infrabony defect measures at different follow-up periods.</i>	<i>Error! Bookmark not defined.</i>

LIST OF TABLES

Table 1	<i>One-Way ANOVA for the effect of different treatment options on the mean of PC concentration</i>	Error! Bookmark not defined.
Table 2	<i>Repeated measures ANOVA shows Effect of follow-up on the mean concentration of PC</i>	69
Table 3	<i>One-Way ANOVA test shows effect of different groups and follow-up periods on mean plaque index:</i>	Error! Bookmark not defined.
Table 4	<i>Repeated measures ANOVA test shows effect of follow up on mean values of PI</i>	73
Table 5	<i>One-Way ANOVA test shows effect of different groups and follow-up periods on MGI</i>	76
Table 6	<i>Repeated measures ANOVA shows effect of follow up on mean values of MGI</i>	77
Table 7	<i>One-Way ANOVA test shows effect of different groups and follow-up periods on mean PD</i>	80
Table 8	<i>Repeated measures ANOVA shows effect of follow up on mean values of PD</i>	81

Table 9	<i>One-Way ANOVA test shows effect of different groups and follow-up periods on mean CAL</i>	84
Table 10	<i>Repeated measures ANOVA shows effect of follow up on mean values of CAL</i>	85
Table 11	<i>One-Way ANOVA test shows effect of different groups and follow-up periods on mean relative intrabony defect measure</i>	87
Table 12	<i>Repeated measure ANOVA shows effect of follow up on mean values of intrabony defect measure</i>	89
Table 13	<i>Summarizing the mean values of drug concentration over the follow-up period</i>	90

INTRODUCTION AND REVIEW OF LITERATURE

Oxygen is a double edged weapon. It is vital to life, however it may have potential toxic effect. Because of its highly reactive nature it is capable of participating in the production of potentially damaging molecules known as free radicals. Free radicals can be defined as being any species have the ability to exist independently while having one or more unpaired electrons as a result they are highly reactive by nature (**Chapple et al., 1997**) (**Pendyala G et al., 2008**).

Reactive oxygen species (ROS) are a subgroup of a larger "free radicals group" that includes reactive oxygen species, reactive chlorine species, reactive nitrogen species (RNS). However, ROS have become more popular than the term free radicals (**Chapple et al., 1997**). ROS can be defined as intermediate oxygen carrying metabolites with or without an unpaired electron, which have the capability of oxidizing other components and converting them into free radicals (**Rammal H et al., 2010**), (**Bouayed J et al., 2010**).

Body cells produce free radicals (oxidants) when they are exposed to substances that induce their production (pro-oxidants). **Dahiya et al, (2013)** classified sources of pro-oxidants into 2 main categories, endogenous and exogenous sources. Endogenous sources were further classified into two mechanisms. The first mechanism includes their production as being by-products of metabolic pathways.

This process occurs within the mitochondria during cellular metabolism by the action of electron transport systems resulting in electron leakage (Free radicals production). Second mechanism is called functional generation in which free radicals are produced by the action of host defense cells (phagocytes) and connective tissues cells. Exogenous sources include heat, smoking, infection, trauma, ultrasound, infection, ultraviolet light, ozone, radiation, exhaust fumes, and therapeutic drugs.

Free radicals play a dual role. They are essential for many biologic processes in mammalian cells, yet they can have a toxic effect (**Chapple IL et al., 2007**). The sensitive balance between their beneficial and injurious effect is an important aspect of life. At low or moderate ROS levels, they have beneficial effects on cells function and immune system function. At high concentrations, they result in generation of deleterious types of stresses called "Oxidative stresses" that can damage all cell structures (**Valko et al., et al 2007**).

Oxidative stress is a general term first defined by **Sies et al. (1991)** as disturbance in balance between production of reactive oxygen species (ROS) and a biological system's capability of detoxifying these reactive intermediates or repairing the resulting damage. There is an ascending body of evidence in literature to illustrate the role of oxidative stresses in the pathogenesis of different types of inflammatory diseases, including periodontal disease (**Masi S et al., 2011**).

In normal physiology, there is a continuously active equilibrium between activity of ROS and the capacity of antioxidant defense system. When the shift occurs in favor of ROS, either by impairment of anti-oxidant defenses or an elevation in ROS production level or activity, oxidative stress results (**Waddington R et al., 2000**). Antioxidants are defined as substances which when available at low concentrations, in comparison to an oxidisable substrate like DNA, proteins or lipids, will significantly retard or prevent oxidation of that substrate (**Wanasundara and F. Shahidi, 2005**).

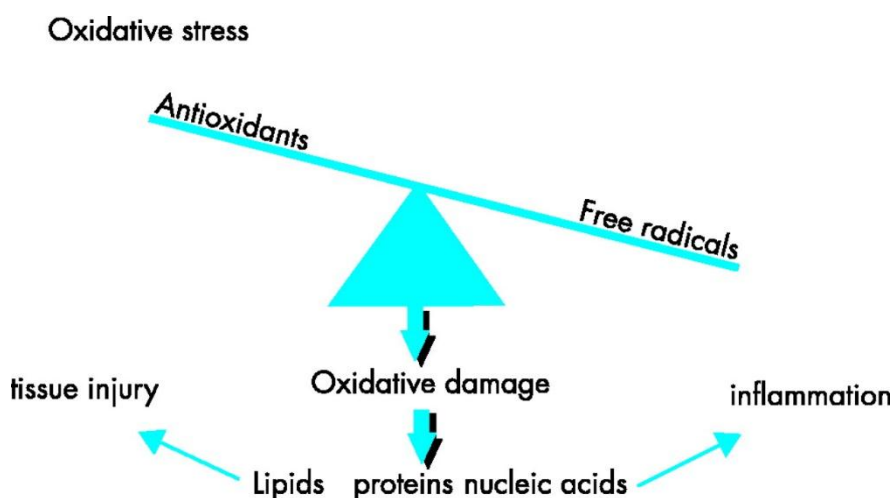


Figure 1. A digramatic representation shows oxidative stresses generation and their effect on different cell structures (**Kelly F. et al., 2003**).

Body cells have different mechanisms to elevate antioxidants level, which can be naturally produced in situ (Endogenous antioxidants) like superoxide dismutase (SOD) and catalase (CAT) or supplied from an external source through diet and/or dietary

supplements (Exogenous antioxidants) like vitamin C and carotenoids (Chatterjee M et al., 2007).

Reactive oxygen species (Oxidants):

During normal cellular metabolism, ROS are generated from molecular oxygen. ROS can be divided into two distinct groups, true radicals and non-radicals. Non-radicals have the ability to produce radicals in the intra-cellular and the extra-cellular environments. True free radicals group involves superoxide ($O_2^{\circ-}$), Hydroxyl (OH°), hydroperoxyl (HOO°) and perhydroxyl ($HO_2^{\circ-}$) (where ‘ $^{\circ}$ ’ denotes an unpaired electron) while non-radicals ROS group involves hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) hypochlorous acid ($HOCl$), and ozone (O_3) (Dahiya et al., 2013).

The most common reactive oxygen species of physiological importance are superoxide anion ($O_2^{\circ-}$), hydroxyl radical (OH°), and hydrogen peroxide (H_2O_2). Superoxide anion is generated by addition of one electron to molecular oxygen (Miller DM et al., 1990). The process which is mediated by the action of nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase and xanthine oxidase or by electron transport system within the mitochondria.

NAD(P)H oxidase is an enzyme found in neutrophils (PMNs), monocytes, and macrophages. After phagocytosis, these cells have the ability to produce a burst of superoxides "Respiratory burst" that leads