" S t u d y p r o t o c o l "

Evaluation of the Efficacy of Topically Applied Melatonin Gel as Adjunctive Therapy in Chronic Periodontitis; (Randomized Control Trail)

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Protocol

Result : **1/1/202o**

Introduction

Periodontal disease is a multifactorial disease. Etiopathogenesis of periodontal disease are not clear. However, it is known that the microorganisms in the mouth start a process and attack healthy tissue. The direct effect of bacterial-derived toxic products and the stimulation of the immunological system with bacterial infection results in damage to the periodontal tissue (1).

Bacterial and products induce inflammatory responses in the tissues, resulting in polymorphonuclear leukocyte migration, differentiation into fibroblasts, activation of macrophages and a number of products such as Interleukin-1 (IL-1), Tumor necrosis factor-α (TNF-α), prostaglandins and hydrolytic enzymes leading to hard and soft tissue destruction (2).

The receptor activation of nuclear factor-kappa B ligand (RANKL) is an important protein in osteoclastic differentiation and proliferation (3). Another protein, osteoprotegerin (OPG), interferes with its biologic potential. RANKL and OPG play critical roles in the development of periodontal disease, with periodontal bone destruction resulting from the upregulation of RANKL with downregulation of OPG (4)

The first treatment goal in periodontal disease is changing or removing microbial origin and risk factors to prevent disease progression and maintain periodontal tissue healt2xh .then ,the recurrence of disease must be prevented and finally, restructured and reorganizing attachment has to be done (5). The knowledge that periodontitis is a chronic inflammatory disease provide us with options to consider using anti-inflammatory agents as therapeutic strategies additional to conventional periodontal treatment andrisk

reduction strategies. This concept has been referred to as host response modulation or host modulation therapy, denoting that the treatment aims to modify the host response by reducing those damaging aspects of the inflammatory response that lead to tissue destruction (6)

Melatonin (N-acetyl-5-methoxy-tryptamine), secreted from the pineal gland and other organs, is an antioxidant and anti-inflammatory agent. Melatonin, first described in 1917, was not

isolated until 1958 (7). Melatonin has been reported to be related to various biological events such as sleep, emotional state, immunity, thermoregulation, sexual maturation and reproduction, especially the circadian rhythm. In addition, it is thought that melatonin, which has been shown to have antiproliferative and antioxidant effects in studies, may be effective in the treatment of cancer and prevention of aging (8).

The inflammatory process may trigger increased plasma melatonin concentration in periodontitis. This leads to an increase in melatonin in the oral cavity and indicating that plays a protective role. There is a significant increase in melatonin level in elderly patients with excessive periodontal insult. Thus, the increase in melatonin in periodontal disease may be secondary to the increase of free radical products in these pathologies (9). An increase in saline melatonin level can affect the periodontal inflammatory process and improve the defensive response of the organism due to the antioxidant and antiinflammatory effects of melatonin (10).

Melatonin strongly suppresses nitric oxide (NO) and interleukin-6 (IL-6) production induced by lipopolysaccharide (LPS) from *P. intermedia*, a major cause of inflammatory periodontal disease, in macrophages (11). The administration of melatonin, in local or systemic form, might be indicated in chronic periodontitis patients, with the goal to protect their mouth against inflammatory and infectious processes of diverse nature.

Aim of Study

The aim of study is to evaluate the efficacy of Melatonin gel as adjunctive to scaling and root planning in the treatment of chronic periodontitis using clinical parameters as well as assess the level of RANKL in gingival crevicular fluid.

Patients and Methods

Study type: randomized clinical trial

Study setting and population

The present study will be carried out as split mouth design on chronicperiodontitis patients; those will be selected from the outpatient clinics of department of Oral Medicine and Periodontology, Faculty of Dental Medicine, Al-Azhar University, Assiut.

Eligibility criteria of population:

Inclusion criteria:-

All chronic periodontitis patients to be included in this study should have pocket depth less than 6 mm.

All patients should be free from any systemic diseases according to criteria of Cornell medical index (12)

Exclusion criteria:-

Smokers Patients, pregnant, received antibiotics or non-steroidal anti-inflammatory drugs within 3 months before the beginning of the study or received periodontal treatment within 6 months before the beginning of study will be excluded.

Ethical considerations:-

The research protocol will be approved by the ethical committee, faculties of dental medicine, Al-Azhar University and the enrolled patients should sign written consent form.

Grouping and intervention:

Patients selected in this study will be classified into 2 groups:

Group 1: twenty chronic periodontitis patients with pocket depth less than 6mm will receive scaling and root planning combined with Melatonin gel adjunctive . Group 2: twenty chronic periodontitis patients with pocket depth less than 6 mm will receive scaling and root planning only

Evaluation:- Clinical evaluation: Clinical parameters including Gingival index (GI) (Löe & Sillness) (13) , Plaque index (PI) (Sillness & Löe) (14) and clinical attachment loss will be taken for all patients at base line 1, 3 months after treatment.

Biochemical evaluation:

The level of RANKL in gingival crevicular fluid will be measured at baseline, 1, and 3 months using ELISA technique.

Data analysis: The data will be collected, tabulated, computed and analyzed using suitable statistical program.

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Results:

Table (1) *Means ± SD of Gingival index ,Plaque index, Pocket depth ,Attachment level , RANKL , paired t- test and P- values in the two groups:*

	Group I Mean±SD	Group II Mean±SD	t- test	P-value
GI				
Baseline	2.8±0.41	2.6±0.5	1.378	0.109
1week	1.6±0.5	1±0	5.339	<0.0001***
1month	0.7±0.47	1.1±0.31	3.183	0.003**
3month	0.8±0.41	1.85±0.37	8.536	<0.0001***
PI				
Baseline	2.5±0.51	2.6±0.5	0.623	0.205
1week	1±0	1±0	6.332	<0.001**
1month	1±0	1±0	6.332	<0.001**
3month	1±0	1.9±0.55	7.285	<0.0001**
PD				

Baseline	4.8±0.41	4.9±0.31	0.872	0.24
1week	4.15±0.67	4.6±0.5	2.401	0.01*
1month	2.55±0.51	3.7±0.47	7.411	<0.0001***
3 month	3.25±0.64	4.3±0.47	5.921	<0.0001***
Attachment level				
Baseline				
	2.8±0.41	2.9±0.31	0.872	0.24
1week	2.15±0.67	2.6±0.5	2.401	0.01*
1month	0.55±0.51	1.7±0.47	7.411	<0.0001***
3months	1.2±0.7	2.3±0.47	5.858	<0.0001***
RANKL				
Baseline	13.89±2.13	15.43±3.53	1.661	0.105
1week	10.56±1.63	12.61±1.96	3.593	0.001**
1month	10.33±0.82	12.81±2.42	4.343	<0.001***
3month	11.84±1.32	13.35±2.05	3.1	0.004**

Table (2) *Correlation Coefficient (r) between RANKL levels & clinical parameter in both groups at different intervals*

RANKLE concentration	Plaque index	Gingival index	Pocket depth	Attachment level
Group I				
Baseline	0.7	0.315	-0.218	-0.218
1 week	0	0.249	-0.453	-0.453
1month	0	0.199	0.148	0.148
3month	0	0.528	0.051	0.107
Group II				
Baseline	-0.568	0	0.406	0.406
1 week				
1month				
	0	0	0.462	0.462
	0	-0.467	0.535	0.535
3month	0.449	-0.061	0.532	0.532