Comparative Evaluation Of Glycemic Status, Levels Of Superoxide Dismutase, Catalase, Lipid Peroxidase And Osteocalcin In Gcf In Type-Ii Diabetes Patients With Chronic Periodontitis Under Ayurvedic Treatment.

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ABSTRACT
Background: Nishamalaki or Nisha Amalaki representing various combination formulations of Turmeric and Indian gooseberry is recommended in Ayurveda, proven efficacious and widely practiced in the management and prevention of complications of Madhumeha (Diabetes).
Aims: To assess the effectiveness of scaling and root planing on the glycemic status and gingival crevicular fluid (GCF) levels of Malondialdehyde, Superoxide Dismutase, Catalase and Osteocalcin in Type II diabetes patients with chronic periodontitis under systemic administration of Nishamalaki, an ayurvedic hypoglycemic formulation.
Material and methods: The study included 90 newly diagnosed Type II diabetic patients in the age group between 30 to 50 years with mild to moderate chronic periodontitis and was randomly divided into 45 patients of two groups each. Group A were Type II diabetic patients with mild to moderate chronic periodontitis taking **supplements of Nishamalaki 1 gram twice daily for 3 months** *and Group B were Type II diabetic patients with mild to moderate chronic periodontitis undergoing scaling and root planing and receiving* **supplements of Nishamalaki 1 gram twice daily for 3 months.**
Results: Independent t-test has been applied to compare the two groups. It has been observed there is no significant difference exists between the two groups. However administration of Nishamalaki and scaling has been all effective in group B parameters.
Conclusion: Nishamalaki showed protective effect and appeared to be useful in reducing the glycemic status, levels of superoxide dismutase, catalase, lipid peroxidase and osteocalcin in GCF in type-2 diabetes patients with chronic periodontitis.

Keywords: Amla, Ayurveda, Diabetes, Nishamalaki, Periodontitis.

1. INTRODUCTION

Periodontitis is one of the most ubiquitous diseases and is characterized by the destruction of connective tissue and dental bone support, which may result in tooth loss; following an inflammatory host response secondary to infection by periodontal bacteria.[1] The destruction of the periodontium is associated with the presence of gram-negative anaerobic bacteria

localized in the sub gingival region, and include typically Porphyromonas gingivalis, Prevotella intermedia, Aggregaterbacter Actinomycetemcomitans, and Tannerella forsythia.[2] These bacteria are considered to play a significant role in the pathogenesis of periodontitis and the formation of the periodontal pocket, destruction of the connective tissue, and resorption of the alveolar bone. Diabetes Mellitus and chronic periodontitis are chronic diseases that have long been considered to be biologically linked. Diabetes patients with poor glycemic control are at greater risk for progression of periodontal destruction over time, and are more likely to have severe periodontitis than those with well controlled diabetes.[3] Increased blood glucose levels in diabetes are reflected in increased levels of GCF glucose. Thus, elevated GCF glucose levels in diabetes may adversely affect periodontal wound healing events and the local host response to microbial challenge. It is well known that oxidative stress plays a major role in the pathogenesis of many diseases, and a defective natural antioxidant status was observed in periodontal diseases and diabetes mellitus. In oxidative stress, the biomolecular damage is caused by attack of reactive species (RS) upon the constituents of living organisms. Most of the biologically relevant RS are either reactive oxygen species (ROS) or reactive nitrogen species (RNS). [4] ROS originate from the incomplete reduction of molecular oxygen resulting in the accumulation of oxidants like hydrogen peroxide (H_2O_2) or free radicals like the hyperoxide (superoxide) anion ($O_2 \cdot -$).[5] They are continuously generated in cells as byproducts of normal aerobic metabolic pathways through the oxidative phosphorylation chain. Reactive nitrogen species includes nitric oxide (.NO), nitrogen dioxide ($NO_2 \cdot -$), and peroxynitrite ($OONO-$).[6] The polymorphonuclear leukocytes (PMNL) are the prime inflammatory cells in gingiva and periodontal tissues. In periodontitis, PMNLs which are naturally capable of producing ROS are thought to be functionally activated and thus lead to increased ROS production. The reactive oxygen species cause periodontal tissue damage by ground substance degradation, collagenolysis directly or indirectly or as a result of oxidation of proteases, stimulation of excessive proinflammatory cytokine release through PG - E2 production via lipid peroxidation and superoxide release, both of which have been linked with bone resorption. Since 1L-1 &TNF-α positively regulate their own production, the additive effects of endotoxin mediated cytokine production and that arising from respiratory burst of PMNLs in response to the same organism, lead to periodontal inflammation and subsequent attachment loss.[7] Various researches have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential.[8] Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. India has a rich history of using various potent herbs and herbal preparations for treating diabetes. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India.[6] There are about 800 plants which have been reported to show antidiabetic potential. A wide collection of plant-derived active principles representing numerous bioactive compounds have established their role for possible use in the treatment of diabetes. Nishamalaki, is a unique ayurvedic formulation, is a combination of *Curcuma longa* (turmeric) and *Amla* (Indian gooseberry). Amla, is perhaps the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Several parts of the plant are used to treat a variety of diseases, but the most important is the fruit. The amla fruit is highly nutritious and contains the high level of heat and storage-stable vitamin ascorbic acid. Extracts from amla fruits have been evaluated for antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive properties. [7-10] Studies have shown than amla interferes with the absorption of the glucose and delays glucose entry in the blood. Thus, it prevents sudden spikes in glucose levels in the blood. Amla inhibits the action of disaccharidase enzyme, which exerts

this action. Furthermore, it has strong antioxidant action, which reduces and prevents the damage to pancreas due to oxidative stress. Amla is helpful for preventing diabetic complications as well. Amla also acts as pancreas stimulant, which increases the secretion of insulin. This property of Amla has similarity with famous anti-diabetic drug glibenclamide. Turmeric is used in Ayurvedic medicine in the treatment of a variety of conditions. The various beneficial activities of Curcuma longa that have been scientifically probed include antioxidant, anticancer, anti-inflammatory, antidiabetic, lipid lowering and wound healing activities among many others. According to studies, curcumin in Turmeric improves the functions of β-cells of pancreas. Nishamalaki prevents frequent infections, protects eyes from diabetic retinopathy, and precludes the occurrence of cardiovascular disorders and nephropathy. It also reduces frequent urination and beneficial in all types of urinary disorders that occurs due to diabetes. Nishamalaki is available in form of powder and tablets. The drug is prepared by mixing equal amount of powder of dried rhizome of turmeric and the powder of dried goose berry fruit. Nishamalaki contains safest ingredients and in such a quantity, which is not known for any side effect or toxicity and is even safe for long-term use (more than a year). Since Nishamalaki contains kitchen ingredients, which can be taken along with any allopathic medicines. For precautionary purposes, a minimum 3 hours gap between Nishamalaki and other allopathic or homeopathic medicines should be kept. [11-15] However, till date no study has been done to find the effect of non-surgical periodontal therapy on oxidative stress markers in Type II diabetes mellitus patients with chronic periodontitis under ayurvedic treatment.

AIM

To assess the effectiveness of scaling and root planing on the glycemic status and gingival crevicular fluid (GCF) levels of Malondialdehyde, Superoxide Dismutase, Catalase and Osteocalcin in Type II diabetes patients with chronic periodontitis under systemic administration of Nishamalaki, an ayurvedic hypoglycemic formulation.

2. METHODOLOGY

The research protocol was approved by the Institutional Human Ethical Committee (IHEC/0022/2015, dated 11.12.2015) before the initiation of the study. All the subjects were informed about the study and written informed consent were obtained prior to the onset of the study. The study included 90 newly diagnosed Type II diabetic patients in the age group between 30 to 50 years with mild to moderate chronic periodontitis and was randomly divided into 45 patients of two groups each.

Group A:
Type II diabetic patients with mild to moderate chronic periodontitis taking supplements of *Nishamalaki* 1 gram twice daily for 3 months.

Group B:
Type II diabetic patients with mild to moderate chronic periodontitis undergoing scaling and root planning and receiving *supplements of Nishamalaki* 1 gram twice daily for 3 months.

A total of 90 male subjects, with newly diagnosed diabetes mellitus patients with chronic periodontitis were enrolled in the clinical trial according to the inclusion and exclusion criteria. Clinical parameters including Plaque Index, Gingival Bleeding Index, Probing Pocket Depth, Clinical Attachment Level, biochemical parameters including the GCF levels of Malondialdehyde, Superoxide Dismutase, Catalase and Osteocalcin and Fasting Blood sugar, microbiological parameters (red complex) namely Porphyromonas Gingivalis, Treponema Denticola, Tanerella Forsythia were assessed at baseline. Following initial

periodontal clinical examination cluster randomization sampling method was applied in which the first 45 subjects received Nishamalaki 1 gram twice daily for 4 weeks, and the remaining 45 subjects undergone scaling and root planning and received Nishamalaki 1 gram twice daily for 4 weeks All the parameters were reassessed at the end of 1st, 2nd, and 3rd months following interventions. All patients were instructed to be on standard dental homecare procedure including use of non-medicated tooth paste with soft to medium tooth brushes, with bass brushing technique twice daily, along with daily drug regimen as a part of glycemic control. Measures were taken to ensure that the Nishamalaki drug was taken periodically.

3. RESULTS

Table no.1 shows the mean and standard deviation of the age of the patients, selected clinical and biochemical variables by group wise at baseline assessment. The purpose of this table is to find out is there any variation exists between the two groups before intervention with respect to the above variables. Independent t-test has been applied to compare the two groups. It has been observed there is no significant difference exists between the two groups except on variables PG, PIA and TF. Even though there is a statistical difference exists between the two groups on PG, PIA and TF, there is no much variation on the mean values. For example, the mean PG value has been 26.58 and 26.34 respectively for group A and group B.

Table No.1: Mean and Standard deviation of age, clinical and biochemical variables at baseline by group wise

Variables	Group A		Group B		t-test value	d.f.	P-value
	Mean	SD	Mean	SD			
Age in years	32.156	1.4609	32.200	1.4397	-.145	88	.885
PI	2.596	.3966	2.618	.3756	-.273	88	.786
GI	1.973	.2189	1.987	.2138	-.292	88	.771
PPD	7.000	.7385	7.044	.7057	-.292	88	.771
CAL	7.667	.6030	7.711	.5886	-.354	88	.724
FBS	132.822	2.2491	132.667	2.3549	.320	88	.749
SOD	51.844	5.2482	52.489	5.1285	-.589	88	.557
CAT	39.022	3.6023	39.489	3.4154	-.631	88	.530
MDA	1.1729	.33842	1.1936	.35977	-.281	88	.780
OSTCA	41.689	3.2810	41.978	3.2856	-.417	88	.677
PG	26.5804	.38890	26.3424	.41712	2.800	88	.006
PI(A)	25.7009	.46397	25.9436	.46787	-2.471	88	.015
TF	24.4604	.42110	24.8064	.45797	-3.731	88	.000

Table No.2 : Comparison Between Group A and Group B of PI

2X4 ANOVA repeated test result			Repeated Contrast test result		
Source	F-value	P-value	Comparison	F-value	P-value
Between Subjects					
Group	109.433	<0.001			

Within Subjects					
Assessment	313.745	<0.001	Baseline vs First	428.195	<0.001
			First vs Second	128.569	<0.001
			Second vs Third	60.635	<0.001
Assessment * Group	333.901	<0.001	Baseline vs First	533.864	<0.001
			First vs Second	128.569	<0.001
			Second vs Third	60.635	<0.001

In Table no. 2, since Group A intervention is not effective in controlling the PI values and Group B is effective in controlling the GI values. The significant p-value of the "Between Subjects Group" comparisons infers that the two interventions A and B are statistically different on controlling the PI Values.

Table No.3 A: Comparison Between Group A and Group B of GI

2X4 ANOVA repeated test result			Repeated Contrast test result		
Source	F-value	P-value	Comparison	F-value	P-value
Between Subjects					
Group	864.292	<0.001			
Within Subjects					
Assessment	645.603	<0.001	Baseline vs First	861.102	.<0.001
			First vs Second	7.918	0.006
			Second vs Third	31.759	<0.001
Assessment * Group	1799.828	<0.001	Baseline vs First	1290.623	<0.001
			First vs Second	375.041	<0.001
			Second vs Third	508.150	<0.001

In Table no. 3, since Group A intervention is not effective in controlling the GI values and Group B is effective in controlling the GI values. The significant p-value of the "Between Subjects Group" comparisons infers that the two interventions A and B are statistically different on controlling the GI Values. The corresponding "Repeated Contrast test" results are also ensuring the above findings. Hence it is concluded Group B intervention is required to treat the GI level of the Periodontitis patients.

The Mean PPD value has been 7.04, 5.97, 5.0 and 3.40 respectively at baseline, First month, Second month and Third month assessments. Results concluded the Group B-intervention has been effective in treating the PPD level of the periodontitis patients. The Mean CAL value has been 7.71, 6.62, 5.60 and 3.33 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the CAL level of the periodontitis patients. The Mean FBS value has been 132.66, 129.60, 126.22 and 118.55 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in

treating the FBS level of the periodontitis patients. The Mean SOD value has been 52.48, 59.97, 61.95 and 62.28 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the SOD level of the periodontitis patients. The Mean CAT value has been 39.48, 47.33, 49.53 and 49.75 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the CAT level of the periodontitis patients. The Mean MDA value has been 1.19, 1.13, 1.05 and 0.37 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the MDA level of the periodontitis patients. The Mean OSTCA value has been 41.97, 49.60, 51.95 and 59.73 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the OSTCA level of the periodontitis patients. The Mean PG value has been 26.34, 27.34, 28.33 and 29.44 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the PG level of the periodontitis patients. The Mean PIA value has been 25.94, 26.94, 27.99 and 28.96 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the PIA level of the periodontitis patients. The Mean TF value has been 24.80, 25.82, 26.85 and 27.83 respectively at baseline, First month, Second month and Third month assessments. Hence, it is concluded the Group B-intervention has been effective in treating the TF level of the periodontitis patients.

4. DISCUSSION

Nishamalaki is an Ayurvedic formulation prepared from Amla (Emblica officinalis) and Turmeric (Curcuma longa). Traditional use mentioned its utility to control DM in initial phase and continued with addition of other agents for maintenance. Individual agents in Nishamalaki show hypoglycaemic, anti-oxidant and neuro-protective effect. Hence with randomized controlled clinical trial an attempt was made to assess the effectiveness of systemic administration of Nishamalaki on the glycemic status and gingival crevicular fluid (GCF) levels of Malondialdehyde, Superoxide Dismutase, Catalase and Osteocalcin in Type II diabetes patients with chronic periodontitis with and without scaling and root planning. These results are consistent with the study of Sharma et al., showing antioxidant action of curcumin responsible for the protective effect in-nephropathy [10] and the raised level of serum creatinine and urea nitrogen in the aged rats were decreased following the administration of E. officinalis extract and reduced age related renal dysfunction by oxidative stress in rats [11] Kasabari et al., showed E. officinalis insulin mimetic effect and also demonstrated inhibitory effects on α-amylase and α-glucosidase. [12] Curcuma has got protective role when used prophylactically and protects islets against streptozotocin-induced oxidative stress by scavenging free radicals effectively rescue islets from damage with no effect on normal cell function.[13] Nishamalaki delayed maturation of diabetic cataract due to slow progression. Although onset of cataract due to STZ-induced hyperglycaemia was not affected, progression and maturation were delayed significantly in a prophylactic and therapeutic Nishamalaki group. The protective effect was even more pronounced with prophylactic group. Our findings about the cataract development in Nishamalaki are consistent with the Suryanarayana et al., that turmeric delays STZ-induced cataract.[14,15] Histopathology of kidney showed tubular cell necrosis, tubular lumen dilation, foci of denuded basement membrane, vacuolization, pyknotic nuclei in diabetic group. With NA & Enalapril, near

normal glomerular and tubular structures compared with the shrunken glomerulus, tubular vacuolations in DM control group. Protective effect may be seen because of the synergistic combination of Curcuma Longa and Emblica officinalis contributing through possible different mechanisms like- increasing Insulin sensitivity, increasing Glucose Uptake, decreasing α glucosidase activity, decreasing Amylase activity, increasing Insulin release, decreasing Oxidative stress. Also Nishamalaki through multiple actions has showed protective effect against diabetic nephropathy and also prevented and delayed development of cataract. Thus, like in our study Nishamalaki appeared to be useful in reducing the glycemic status, levels of superoxide dismutase, catalase, lipid peroxidase and osteocalcin in GCF in type 2 diabetes patients with chronic periodontitis.

5. CONCLUSION

Nishamalaki showed protective effect and appeared to be useful in reducing the glycemic status, levels of superoxide dismutase, catalase, lipid peroxidase and osteocalcin in GCF in type-2 diabetes patients with chronic periodontitis. Nishamalaki administration observed to be more effective in prophylactic groups. Dietary use of curcuma longa and Emblica officinalis show beneficial effects but the scientifically prepared formulations are definitely effective as alternative treatment strategies for prevention of diabetic Periodontitis. For the assessment of exact duration of prophylactic use of drug administration, further studies are required.

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