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Effects of Smoking to the Lipid Profile in Patients with Periodontal Disease.

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ABSTRACT

The aim of this study was to determine the effects of smoking to the lipid status in patients with periodontal disease through analyzing the serum dynamics of lipid parameters (triglycerides, total cholesterol, LDL and HDL cholesterol). The examined group was consisted of 45 patients diagnosed with periodontal disease, who were smokers; the control group was consisted of 45 patients diagnosed with periodontal disease, as well, but non-smokers. All the patients were in the range of age from 40-60 years. According to the number of cigarettes, the patients from the examined group were divided into three sub-groups, each consisting of 15 examinees. The first sub-group was consisted of patients who smoked up to 10 cigarettes in a day (light smokers); the second sub-group was consisted of patients who smoked from 10 to 20 cigarettes in a day (moderate smokers); the third sub-group consisted of patients who smoked more than 20 cigarettes in a day (passionate smokers). The values of Silness-Loe dental plaque index (DPI), index of gingival inflammation (IGI), Cowell gingival bleeding index (GBI) as well as the level of attachment loss (Ramfjord) were noted in both examined and control groups. The results of the total cholesterol and HDL cholesterol showed statistical significant difference between the mean values in the three sub-groups of the examined group, while the mean values of HDL cholesterol in the second and third examined sub-groups and control group are in higher range than the normal one.

Keywords: TNF-α and IL1-β, periodontal indices, lipid status, smoking.

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INTRODUCTION

The risk of periodontal disease and its prognosis is associated with a number of factors like: age, stress, presence of specific microorganisms, genetics, diabetes and smoking (1). Historically, it was believed that all individuals are uniformly susceptible to development of periodontal disease and that it is enough only the accumulation of the dental plaque, bad oral hygiene and probably the occlusal trauma to exist, for the periodontal disease to be initiated. In last four decades it has been accepted that the periodontitis is caused by specific bacterial infection and the individuals are equally susceptible to these infections and the damage that they cause. This understanding made the clinicians and researches to focus their efforts to develop markers which will help to identify the susceptible individuals, just before the initiation of the disease, as well as the risk factors which can be modified in order to prevent the periodontal disease or change its course (2). Smoking is the best confirmed modifying risk factor in the developing and progress of periodontal disease (3). Smoking can be involved in the etiopathogenesis of the periodontal disease through releasing pro-inflammatory cytokines and inflammatory mediators which can be able to initiate cascade of biochemical reactions and to cause periodontal and endothelial damage (4). Thus, smokers can be systemically affected even in absence of clear clinical symptoms of the disease (5).

Aim of the study

Taking into consideration the literature data associated to the influence of the smoking to the systemic health, as well as to the pathogenic mechanisms of the periodontal disease, the aim of the study was set: to determine the effects of smoking in patients with periodontal disease, through analyzing the serum dynamics of the lipid parameters (triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol)

MATERIALS AND METHODS

In order to achieve the set aim, an examined group consisted of 45 patients diagnosed with periodontal disease, who were smokers and a control group consisted of 45 patients with diagnosed periodontal disease, which were non-smokers, were formed in the Clinic for periodontology and oral medicine. All the patients were in the range of age from 40-60 years. The periodontal disease that was present was classified according to the American Association of Periodontology from 1999. The diagnosis was set on basis of anamnesis, clinical evaluation and x-ray findings. A great attention was paid to the anamnesis, in order to gain detailed data for verification of absence of any systemic disease. All the patients signed an informed consent and agreed that the collected data and materials would be used only for scientific and research purposes. According to the number of cigarettes, the patients from the examined group were divided in three sub-groups, each consisting of 15 examinees, as follows:

- first sub-group, consisted of patients who smoked up to 10 cigarettes in a day (light smokers)
- second sub-group, consisted of patients who smoked from 10 to 20 cigarettes in a day (moderate smokers)
- third sub-group, consisted of patients who smoked more than 20 cigarettes in a day (passionate smokers)

The values of Silness-Loe dental plaque index (DPI), gingival inflammation index (IGI), Cowell gingival bleeding index (GBI) as well as the level of attachment loss (Ramfjord) were noted in both examined and control groups. After the clinical evaluation and index values determination, a venepuncture of the cubital vein was performed in all the examinees from both groups and 5 ml of blood were collected from each of them. The blood was collected in sterile test-tubes and transported to the Institute of Physiology in the Medical Faculty in Skopje. The samples were remained still for 2 hours, at room temperature. After the coagulum retraction, the remained serum was centrifuged using Becman centrifuge in 5000 rotations per minute. The analysis of the lipid status was performed using the colorimetric method (Merck diagnosis).

RESULTS AND DISCUSSION

Smoking and its duration lead to increased concentration of total serum cholesterol, triglycerides, LDL cholesterol, but to lower anti-atherogenic HDL cholesterol (6), which plays a key role in atherosclerosis process. The combination of nicotine and lipopolysaccharide (LPS) can result in releasing inflammatory

cytokines such as IL-1β and TNF-α, which influence to the lipid metabolism and promote hyperlipidemia (7). Related to the potential association between smoking, hyperlipidemia and periodontal disease, today it is thought that smoking may induce changes in the immunocellular function, resulting in impaired metabolic regulation of the lipids, through mechanisms which involve proinflammatory cytokines (8).

A considerable number of cytokines, like IL-1β and TNF-α are produced as a result of the presence of periodontal pathogenic gram negative bacteria (9, 10). These cytokines have a great influence to the lipid metabolism (11), either through provoking production of other cytokines and changing the chemodynamics (utilization of amino-acids) from different tissues which participate in the lipid metabolism, or through modifying the hypothalamus-hypophysis-adrenal axis and increasing the concentration of adrenocorticotropic hormones, cortisol, adrenalin, noradrenalin and glucagon in the plasma (12). Therefore, with the help of the activity of IL-1β and TNF-α, microbial exposure and action of nicotine, increasing of the level of free fatty acids, cholesterol and triglycerides occur (13).

The results of this study regarding to the total cholesterol, show statistically significant differences between the mean values of the groups (p = 0.018072) (Table 1), but the mean values are higher in the second and third sub-group in the examined group (Table 2). Post hoc Tukey's test showed predominant difference between the third sub-group and other sub-groups and control group (Table 3), which confirms the influence of the higher number of smoked cigarettes daily to the systemic and periodontal health. The results regarding to the level of total cholesterol in smokers and non-smokers with periodontal disease are in accordance with the results reported by Katz et al. (12) and Loeshe et al. (14), but opposite to those reported by Kenney et al.(16).

Smoking, poor nutrition and fat reach meals result in prolonged impairment of the antibacterial function of the polymorphonuclear leukocytes (16), i.e. hyperreactivity and increased production of oxygen species (50), which is associated with periodontal disease progression in adults.

The concentrations of pro-inflammatory cytokines TNF-α, IL-1β, prostaglandin E2 (PGE-2) reach high levels in individuals with periodontal disease (18). Inflamed periodontal tissues may act as a permanent renewable container for releasing of TNF-α, IL-1β and PGE-2 in circulation, thus evoking extended systemic effects, as well as influencing the lipid metabolism (19). Increasing of serum lipids occurs due to the higher synthesis or lower degradation of triglycerides (9), as well as reduced elimination of LDL cholesterol.

The results regarding to LDL cholesterol show higher values compared to the referent values in the three sub-groups and control group (Table 4). The difference between mean values of the examined groups is not statistically significant for p=0.090300 (Table 5). These results about the level of LDL cholesterol in smokers and non-smokers with periodontal disease are in accordance with the results reported by Katz et al. (12) and Loeshe et al. (14), but opposite to those reported by Kenney et al.(16). Nutrition can influence the host inflammatory response, i.e. to participate in the activation of the inflammatory cytokines that affect the immune function and probably have effect to the periodontal health and the condition of some specific systems in the body (20). The nutrition with higher intake of saturated fat, but lower intake of cellulose and fruits can lead to changes of the lipid status.

The mean values of HDL cholesterol are in the referent ranges in the second and third sub-group and control group, while the mean value in the first sub-group is higher than the referent values (Table 6). The difference between the mean values in the examined groups is statistically different for p=0.000000. According to post hoc Tukey's test the difference is mainly significant between the first and other groups (Table 7). Higher levels of HDL cholesterol show anti-inflammatory action and lower the adhesion of the endothelial cells, with this lowering the risk of cardiovascular diseases. The results regarding to the HDL cholesterol levels in smokers and non smokers with periodontal disease are in accordance with the results reported by Cutler at al. (6), but opposite to those reported by Buhlin et al (15).

Different mechanisms that lead to lipid alteration due to smoking include the action of nicotine which stimulates the sympathetic adrenal system, which results in higher secretion of catecholamines, higher lipase levels and higher concentration of plasm fatty acids and thus, higher secretion of hepatal fatty acids and triglycerides (11). The mean values of triglycerides in the three sub-groups and control group were higher than

the referent ones (Table 8). The difference which can be noted between the mean values in the examined groups (smokers) is not statistically significant (p=0.345041)(Table 9). The biologic signal molecules from the local inflamed tissue has physiologic effects to the stimulation of lipogenesis, increasing the lipolisis and decreasing the lipid clearness, resulting in hyperlipidemia or accumulation of free fatty acids (FFA) and triglycerides (21).

The results regarding to the level of triglycerides in smokers and non-smokers with periodontal disease are in accordance to those reported by Loeshe et al. (14), but opposite to those reported by Buhlin et al (15). The analysis of lipid status in both examined groups showed higher values in both examined groups, which confirms our results (22) of the dependence between the hyperlipidemia and periodontal disease.

The clinical and radiological findings of the periodontal condition indicate that in smokers it is worse compared to non-smokers, while the clinically it is presented with presence of deep periodontal pockets, higher attachment loss, gingival recession, increased alveolar bone loss and higher values of dental plaque (23). Smoking influences the composition of the subgingival bacterial flora as well, with that increasing the subgingival infection. Smoking also has effects to the oxidative-reduction potential of the dental biofilm, creating anaerobic conditions and predomination of gram negative anaerobic bacteria (24). The decreased protective and reparatory capability of the periodontium and the presence of aggressive bacteria in the dental plaque lead to increased damaging of the periodontium in smokers, compared to non-smokers (25, 26). The analysis of dental plaque index (DPI) showed percentual difference between the first and second, compared to the third sub-group which is statistically significant (p=0.0353); the difference between the first and second sub-group compared to the control group is statistically significant (p=0.0354)(Graph 1). The results regarding to the values of the dental plaque index are in accordance with those reported by Machuca et al. (23), but opposite to those reported by Baab and Qberg (27).

According to us, the higher volume of dental plaque in smokers is due to the bad oral hygiene and formation of nicotine pigmentations which increase the plaque accumulation (25, 26). The inflammatory response induced by the accumulation of dental plaque may be modified by the secondary products of the tobacco, like the cotinine (27), a secondary product of the nicotine which has effect of peripheral vasoconstriction and reduces the clinical signs of gingival inflammation, the redness and swelling (28). The reduced intensity of the gingival response is probably due to vascular changes; the thickness of the marginal gingival epithelium is damaged by smoking. The local vasoconstriction effect of the nicotine leads to lower blood flow in the gingival tissue, hypoxia and decreased capability in elimination of the products of the tissue metabolism (29). All these events have an effect of decreasing the reparatory capability of the periodontium, which clinically is manifested as delayed tissue healing. The analysis of the index of gingival inflammation (IGI) and gingival bleeding index (GBI) indicate to clear percentual difference between the control group and second and third sub-group, which is statistically significant (p=0.0000), and between the control group and third sub-group, where a statistically significance is present (p=0.0036)(Graph 2). This confirms the peripheral vasoconstriction effect of the nicotine, which reflects with low clinical signs of inflammation in smokers.

The results regarding to the values of IGI and GBI are in accordance to those reported by Johnson et al. (30), but there is no literature data which decline this finding. The effect of smoking to the periodontium is cumulative, t.e. the negative effects depend on the duration of smoking and number of smoked cigarettes (31). The alveolar bone loss and attachment loss in smokers are increased, and the correlation depends on the dose of nicotine taken by smoking and the effect showed after years (26, 31). The analysis of attachment loss in the range of 3-6 mm showed percentual difference between the control group and third sub-group which is statistically significant (p=0.0496); statistically significance was found between the control, second and third sub-group for the attachment loss higher than 6 mm (p=0.0496). The percentual difference was not statistically significant (p=0.082)(Graph 4) in the first sub-group and control group for attachment loss up to 3 mm. These results regarding to the index of attachment loss are in accordance with those reported by Rivera-Hidalgo (26) and Tanur et al. (31) , but opposite to those reported by Baab and Qberg (27).

According to us, the nicotine stimulates the osteoclast differentiation, with that increasing the resorption of calcium phosphate, the major structure of bones. Higher concentrations of nicotine lead to increased number of osteoclasts, cells responsible for resorption and remodelation during the periodontal disease.

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Table 1. Mean values of total cholesterol in examined group with its sub-groups and the control group.

group	Mean value	Number	± St.Dev	minimum	maximum
I	5,107143	15	0,523985	4,6	6,7
II	6,080000	15	1,044851	4,9	8,4
III	7,033333	15	2,859987	4,0	13,5
IV	6,270732	45	1,379718	3,5	9,0

This table shows the mean values of total cholesterol which are in normal ranges (3.1-5.5 mmol/l) in first sub-group, while the mean values in other two sub-groups and control group are higher than the normal values.

Table 2. Analysis of variance of the mean values of total cholesterol

SS	df	MS	SS	df	MS	F	p
27,52450	3	9,174834	209,5115	81	2,586562	3,547116	0,018072

Analysis of variance of the mean values of total cholesterol in the examined group with its sub-groups which are statistically significant for p=0.018072.

Table 3. Post hoc Tukey HSD test for total cholesterol

group	I	II	III	IV
I		0,369058	0,009796	0,098171
II	0,369058		0,371497	0,979334
III	0,009796	0,371497		0,400672
IV	0,098171	0,979334	0,400672	

There is statistical significance regarding to the total cholesterol mainly between the third sub-group and other groups.

Table 4. Mean values of LDL cholesterol in examined group with its sub-groups and control group.

group	Mean value	Number	± St.Dev	minimum	maximum
I	4,071429	15	1,188036	1,7	5,2
II	4,620000	15	0,829113	2,4	5,7
III	4,900000	15	2,592572	0,7	9,6
IV	3,821951	45	1,343040	1,3	6,1

This table shows the mean values of LDL cholesterol in examined group with its sub-groups and control groups which are higher than the referent values (2,2 - 3,5 mmol/l)

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Table 5. Analysis of variance of mean values of LDL cholesterol

SS	df	MS	SS	df	MS	F	p
16,08424	3	5,361414	194,2228	81	2,397813	2,235961	0,090300

This table shows the difference between the mean values in examined groups which is statistically significant for p=0.090300

Table 6. Mean values of HDL cholesterol

Group	Mean value	Number	± St.Dev	minimum	maximum
I	3,215385	15	1,302463	1,1	4,9
II	1,446667	15	0,417247	0,5	2,0
III	1,400000	15	0,311677	0,8	1,9
IV	1,368293	45	0,329726	0,7	1,9

This table shows the mean values of HDL cholesterol in examined groups with its sub-groups and control group which are in normal ranges (2.2-3.5 mmol/l) in the second and third sub-group, while the mean values in first sub-group is higher than the referent values.

Table 7. Analysis of Variance of mean values of HDL cholesterol

SS	df	MS	SS	df	MS	F	p
36,61934	3	12,20645	28,50304	80	0,356288	34,26006	0,000000

This table shows the difference between the mean values of HDL cholesterol in examined groups and control group, which is statistically significant (p=0.000000).

Table 8. Post hoc Tukey HSD test

Group	I	II	III	IV
I		0,000147	0,000147	0,000147
II	0,000147		0,996576	0,972293
III	0,000147	0,996576		0,998116
IV	0,000147	0,972293	0,998116	

This table shows the post hoc Tukey HSD test which shows significance mainly between the first sub-group and other groups.

Table 9. Mean values of triglycerides

Group	Mean value	Number	± St.Dev	minimum	maximum
I	2,585714	14	0,799863	1,9	4,5
II	2,453333	15	0,604586	1,2	3,2
III	3,106667	15	1,007448	1,6	5,0
IV	2,521951	41	1,393828	0,9	4,7

This table shows the mean values of triglycerides in the examined group with its subgroups and control group which are higher than the referent ones (0.1-2.2 mmol/l)

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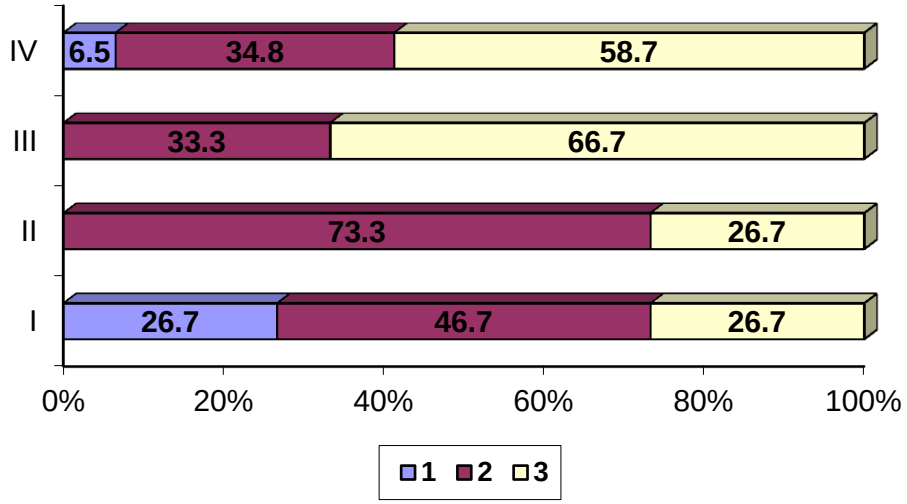
Table 10. Analysis of variance of mean values of triglycerides

SS	df	MS	SS	df	MS	F	p
4,378888	3	1,459629	105,3541	81	1,300667	1,122216	0,345041

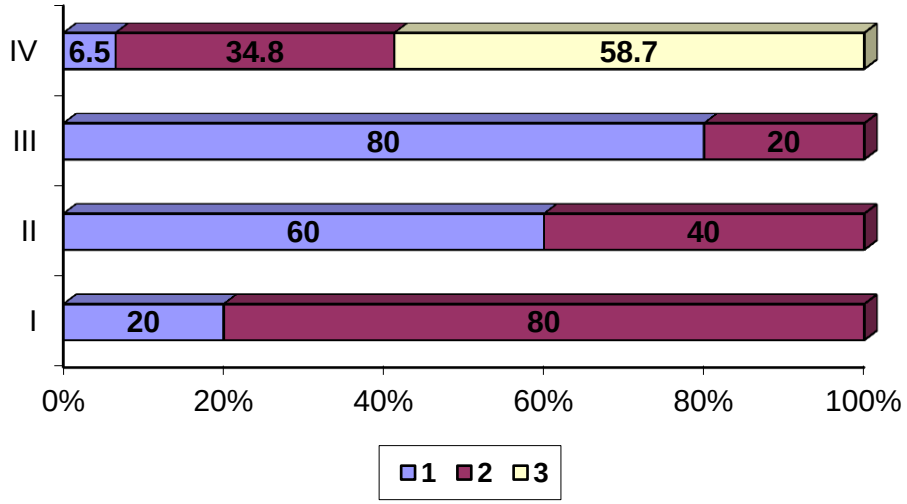
This table shows the difference in the mean values of triglycerides between examined groups and control group which is statistically significant (p=0.345041).

Graph 1. Distribution of Silness-Loe dental plaque index



Graph 1 shows the distribution of dental plaque index in examined group with its sub-groups and control group. There is percentual difference between first and second, compared to the third sub-group which is statistically significant (p=0.0353) and between the first and second sub-group compared to the control group (p=0.354).

Graph 2. Distribution of Silness-Loe index of gingival inflammation



Graph 2 shows the distribution of the index of gingival inflammation in the examined group with its sub-groups and the control group. A clear percentual difference can be noted between the control group and the second and third sub-groups which is statistically significant (p=0.0000) and between the control group and third sub-group (p=0.0036).

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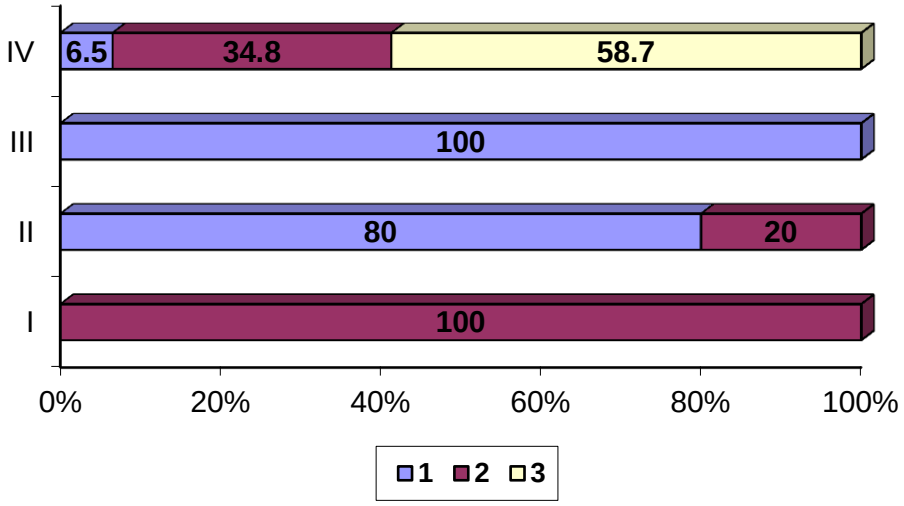
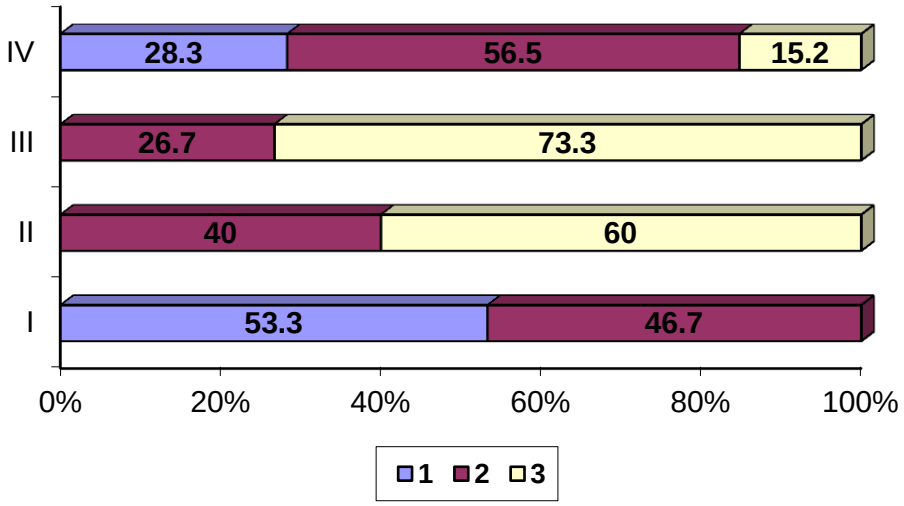
Graph 3. Distribution of Cowell index of gingival bleeding



Graph 2 shows the distribution of the Cowell index of gingival bleeding in the examined group with its sub-groups and control group. A clear percentual difference can be noted between the control group and the first, second and third sub-groups which is statistically significant (p=0.0000)

Graph 4. Distribution of Ramfjord attachment loss index



Graph 4 shows the distribution of Ramfjord attachment loss index in the examined group with its sub-groups and control group. Next findings should be noted:

1- attachment loss up to 3 mm. Percentual difference is not statistically significant (p=0.082).

2- attachment loss from 3-6 mm. Percentual difference is statistically significant only between the control group and third sub-group (p=0.0496).

3- attachment loss higher than 6 mm. Percentual difference is statistically significant between the control group and second and third sub-group (p=0.00).

CONCLUSIONS

The investigation of the role of smoking as a risk factor in etiopathogenetic events in periodontal disease, with verification of the lipid status and clinical parameters, leaded to these conclusions:

1. The parameters of lipid status detected difference between the mean values in the examined groups which are not statistically significant for LDL cholesterol (p=0.090300) and for triglycerides

(p=0.345041), with mean values higher than referent ones in the examined sub-groups and control group, with exception of the values of triglycerides in the first sub-group which are in normal range.

2. The analysis of total cholesterol and HDL cholesterol showed difference of the mean values for HDL in the three examined sub-groups, which is statistically significant (p=0.018072); statistical significance was found between the same groups for the total cholesterol, as well (p=0.000000); mean values of HDL in second and third sub-group and control group are in normal ranges, while the mean value in the first sub-group is higher than referent values.
3. Results from the analysis of the dental plaque index (DPI) showed percentual difference in the first and second, compared to the third sub-group, which is statistically significant (p=0.0353) as well as in the first and second sub-group, compared to the control group (p=0.0354).
4. Analysis of the index of gingival inflammation (IGI) indicates a clear percentual difference between the control group and second and third sub-group, which is statistically significant (p=0.0000), as well as between the control group and third sub-group (p=0.0036).
5. Analysis of the gingival bleeding index (GBI) indicates a clear percentual difference between the control group and first, second and third sub-group, which is statistically significant (p=0.0000).
6. Attachment loss in smokers is increased, with the loss level depends on the nicotine dose taken by smoking (number of smoked cigarettes daily) and effect showed years later.
7. It can be concluded that a very serious approach in the treatment of periodontal disease is needed. It should include frequent check-ups of patients and comprehensive instructions for maintaining oral hygiene when predisposing factor exists.

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