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Research Article

Cellular immunity indexes after therapeutic use of immunoactive and anti-inflammatory agents in patients with orthodontically induced gingival hyperplasia

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Abstract

Orthodontic treatment with fixed appliances that is mediated by the power forces of the apparatus might lead to changes in the blood circulation, predispose to a number of complications such as tooth movement and contributes to the morphological bone tissue remodeling. We have investigated 126 patients aged 16–35 years after appliance bracket therapy that has caused orthodontically induced gingival hyperplasia. Immunological investigation which comprised the study of the cellular immune system in patients with orthodontically induced gingival hyperplasia suggested that there were significant disorders of the cellular immune system that correspond to the T-suppressor type of the secondary immunodeficiency state. Endogenous interferon composed of a low molecular weight organic compounds and an interferon-inducing agent (isonicotinic acid derivative) was used in the treatment of the patients with orthodontically induced gingival hyperplasia. Interferon provides restoration of functional activity in T-lymphocytes, enhances the functional capacity of the macrophages, provides normalization of the cell immunity, contributes to the elimination of secondary immunodeficiency and restoration of immunological homeostasis in individuals with orthodontically induced gingival hyperplasia caused by fixed appliances.

Keywords

Anti-inflammatory agent, gingival hyperplasia, Cellular immunity, immunostimulants

Introduction

According to data researchers (Alimskiy and Dolhoarshynnykh 2008; Anokhyna and Haliulina 2008) orthodontic fixed appliance technique allows to achieve stable morphological results, improve personal appearance not only in children and adolescents, but in adults as well. However, orthodontic treatment in adults with fixed appliances in particular, can predispose to deterioration in periodontal health (Benoist et al. 2007; Holovko and Babenko 2010).

Orthodontic treatment with fixed appliances generates high forces that have ability to promote changes in blood circulation, tooth movement and bone remodeling. On one side, the findings provided by authors (Mashchenko 2003; Herbert et al. 2008) demonstrated that the problem with fixed appliances is a plaque accumulation and decalcification around the brackets, quick development of the

lesions with progressive mineral loss. On the other, they often promote gingival inflammation that is a frequent well recognized complication (Bondareva and Valyeva 2001; Al-Nimri et al. 2009). The actuality of the problem is also concerned with the significant resistance of orthodontically induced gingival hyperplasia (OIGH) to conservative therapeutic use in the treatment (Kyrhyzova and Persin 2008).

The article demonstrates the results of cellular immunity correction in orthodontic patients with OIGH associated with fixed appliances after therapeutic use of immunoactive and anti-inflammatory drugs.

Materials and methods

A total of 126 patients aged 16–35 years affected by OIGH associated with bracket technique were enrolled in this study and were divided into two groups. The main group and comparison group consisted of 63 individuals each. Both groups were randomly arranged by age, gender and severity of OIGH. All patients were appointed with periodontal therapy that comprised removal of both supra- and subgingival deposits with Gracie curettes that provide a thorough mechanical cleaning due to their curved design and a minimal possibility of the gum injury. When scaling the gingival pockets were washed with 0.12% chlorhexidine bigluconate solution. After hand instrumention the directed ultrasound therapy was administered followed by polishing with hydroxiappatite paste providing a smooth tooth surfaces, elimination of the biofilm, excision of granulations from the inner wall of the false pockets.

All patients were instructed on oral hygiene to maintain an adequate standard of plaque control and to improve individual oral hygiene. The patients were introduced with oral hygiene aids such as toothbrushes, interproximal brushes, irrigators. The use of mouthwashes is also essential for the chemical control of plaque, therefore they were appointed with 0.12% chlorhexidine bigluconate to be used twice a day rinsing for 60 seconds during 5–7 days. Reassessment of oral hygiene was carried out by every appointment together with explaining the role of oral plaque, thus to motivate the patient to improve oral hygiene in general.

The patients from the main group were administered with drugs "Cycloferon", "Erbisol" and "Amizon" according to the scheme.

Cycloferon (manufactured by LLC "Scientific and Technological Pharmaceutical Company" POLISAN, Russian Federation; with a registration number: UA/7671/02/01). Each vial (2 ml) contains Acridonoacetic Acid 0.25 g. The drug itself is a low molecular weight inducer of endogenous interferon which also expresses both anti-inflammatory and immunomodulatory activity.

Immunostimulator "Erbisol" (manufactured by ERBIS LLC, Ukraine with a registration number: № UA/9178/01/01 dated 11.12.2019) was also appointed in management. One ml of the drug contains a complex of natural low-molecular organic non-hormonal compounds, obtained from animal embryonic tissue, contains oligopeptides and glycopeptides (generally 0.07–1.0 mg), nucleotides and amino acids. The pharmacological activity of the drug is determined by the content of low molecular weight biologically active peptides, which activate the natural, evolutionarily formed control systems of the body that are responsible for finding and eliminating of the pathogen. Erbisol activates the immune system to accelerate the recovery of the damaged and destruction of abnormal cells and tissues. The main immunomodulatory effect of the drug is manifested first of all via the action on the macrophage link responsible for the reparation of the damaged cells and restoration of functional activity in organs and tissues. The same effect is also demonstrated through NK cells (CD3-16 + 56 +) and T killers (CD3 + 16 + 56 +), which are responsible for the destruction of damaged incapable for regeneration cells or abnormal mutant, malignant, virus-carrying cells. Erbisol, at the same time, provides immunocorrective effect and in case of immune disorders contributes to their normalization due to the activation of T-lymphocytes, Th1-helpers and T-killers and inhibition of the activity in Th2-helpers and B-lymphocytes, which is important for restoring the balance between cellular and humoral immunity in case of cancer and for suspension of allergic reactions. Depending on the body immunity the drug also adjusts the activity of some other factors of humoral and cellular immunity: induces the synthesis of α-, β- and γ-interferon, tumor necrosis factor, interleukin 2 (IL-2) and IL-12, inhibits the synthesis of IL-4 and IL-10).

Amizon (manufactured by JSC "Farmak", Ukraine; with a registration number: № UA/6493/01/01 dated 28.04.2017) was also appointed in management of OIGH. Amizon is a derivative of isonicotinic acid. Each tablet contains amizon (enisamium iodide) 250 mg (0.25 g). This pharmacological agent has an inhibitory effect on influenza viruses, exhibits interferonogenic properties, increases the body's resistance to viral infections. The immunoactive and bactericidal qualities of this drug have also been established especially towards periodontopathogenic obligate anaerobes. Amizon (AID) provides anti-inflammatory effect as a result of stabilization of the cell and lysosomal membranes, slowing down degranulation of basophils, antioxidant action, normalization of prostaglandins, cyclic nucleotides and energy metabolism in the inflammatory focus.

The drugs were administered according to the following scheme: 12.5% solution of Cycloferon 2.0 ml intramuscularly once a day for 5 days, and then 5 more injections a day; after the course of Cycloferon, the second agent Erbisol was administered as follows: 2 ml 1–2 times a day intramuscularly for 15–20 days; Amizon was prescribed by 1 tablet to be taken twice a day for 15–20 days.

We performed analysis to evaluate changes in specific cellular immune related parameters : the count of CD3 +, CD4 +, CD8 + and CD22 + lymphocytes in the venous blood, the lymphocyte blast transformation reaction

(RBTL) and the phagocytic activity of monocytes (PAM) in the peripheral blood.The count of T- (CD3 +) and B-lymphocytes (CD22 +), subpopulations of T-helpers / inducers (CD4 +) and T-suppressors / killers (CD8 +) was studied in a cytotoxicity test using monoclonal antibodies (moAb). Commercial (moAb) classes CD3 +, CD4 +, CD8 +, CD22 + used by MedBioSpectrum (Moscow, Russia) were employed in the research. In this case, moAb T class CD3 + was considered relative to the total population of T-lymphocytes, CD4 + - to the population of T-helpers / inducers, CD8 + - to T-suppressors / killers, CD22 + - to B-cells. Immunoregulatory index CD4 / CD8 was calculated, which was interpreted as the ratio of lymphocytes with helper and cytotoxic activity (Th / Ts).The functional activity of T-lymphocytes was analized by RBTL when setting it by micromethod with phytohemagglutinin (PHA) as a non-specific antigen.

Studies of the monocyte phagocytosis (PAM) were performed by the cup method. Live daily culture of Staphylococcus aureus , strain 505, obtained from the Pasteur Institute (St. Petersburg, Russia) was used as a test object. The following indexes were calculated: phagocytic index (PI) – the percentage of phagocytic monocytes, phagocytic number (PN) – the number of engulfed bacterial cells per 1 monocyte , attraction index (IA) – the number of microbial cells fixed on 100 monocytes, ingestion index (II) – the percentage of ingested microbial cells absorbed by 100 monocytes.

The results of the investigations were processed on a Pentium IBM personal computer in a Windows XP OC using Microsoft Excel 2003 and Statistica, as well as specially designed programs (Delphi JV).

Results and discussion

The findings of immunological investigation obtained before the treatment in patients with OIGH demonstrated that there were expressed violations of the cellular immunity indices. Moreover, there was no probable difference between the analyzed immunological parameters in the main and comparison group, which suggests that the origin and severity of immune disorders in all patients were the same.

These immune disorders were fundamentally characterized by a significant decrease of the RBTL with PHA index that confirmed the suppression of the functional state in T-lymphocytes as well as T-cell lymphopenia of varying degrees and imbalance of the T-lymphocytes subpopulation.

The latter mainly consisted of a probable decrease in the number of CD4+ cells (circulating in the peripheral blood T-helpers/inducers) relating to a moderate decrease in the number of T-suppressors / killers (CD8 + lymphocytes). However, the count of B-cells (CD22 +) in most cases expressed only a slight tendency to decrease, or corresponded to the lower limit of the norm, as summarized in Table 1.

Table 1. Indexes of cellular immunity in patients with OIGH before treatment (M ± m).

Indexes of cellular immunity		Norm	Groups of patients with OIGH		P
			main (n=63)	comparison (n=63)	
CD3+	%	69.6±1.6	53.4±0.8**	54.0±0.9**	<0.05
	10^9/l	1.3±0.03	0.85±0.01**	0.86±0.01**	0.1
CD4+	%	45.5±1.2	34.1±0.7*	34.7±0.8**	<0.05
	10^9/l	0.86±0.02	0.54±0.01**	0.55±0.01***	0.1
CD8+	%	22.5±0.8	21.7±0.7	22.1±0.6	<0.05
	10^9/l	0.42±0.01	0.35±0.01*	0.36±0.01*	0.1
CD22+	%	21.6±0.9	20.6±0.5	21.2±0.7	<0.05
	10^9/l	0.41±0.02	0.33±0.01	0.34±0.01*	0.1
CD4/CD8		2.02±0.03	1.57±0.02***	1.57±0.03***	0.1
RBTL with PHA, %		65.5±2.2	50.1±1.5**	50.9±1.7***	<0.05

* – P < 0.05 – the probability of divergences regarding the norm.
** – P < 0.01 –the probability of divergences regarding the norm.
*** – P < 0.001 – the probability of divergences regarding the norm.
Column P –degree of probability between indexes of the main group and the comparison group.

Thus, the changes of cellular immunological parameters before treatment corresponded to the T-suppressor type of secondary immunodeficiency (SIDS).

Our investigation indicated that the T-lymphopenia and imbalance of the subpopulation of T-lymphocytes were eliminated in most examined, the level of circulating in the peripheral blood T-helpers/inducers (CD4 +) has also increased, which led to normalization of the immunoregulatory index CD4/CD8 in patients of the main group after appointed therapy. Therefore, positive changes in immune indexes suggest that the patients benefit from administered immunotherapy combined with antiinflammatory agent. Analyses demonstrate that there was an improvement of RBTL with PHA associated with restoration of functional activity in T-lymphocytes (Table 2).

Patients from the comparison group appointed with conventional therapy also demonstrated positive changes of the cellular immune related parameters. However, the degree of defined changes in this group was significally lower than in the main group. Consequently, there were notable differences of cellular immunity indexes in comparison group in relation to the norm and to those from the main group (Table 2).

Table 2. Indexes of cellular immunity in patients with OIGH after treatment (M ± m).

Indexes of cellular immunity		Norm	Groups of patients with OIGH		P
			main (n=63)	comparison (n=63)	
CD3+	%	69.6±1.6	67.8±0.9	57.5±0.6*	<0.05
	10^9/l	1.3±0.03	1.22±0.02	0.97±0.01*	<0.05
CD4+	%	45.5±1.2	45.4±0.8	38.9±0.7*	<0.05
	10^9/l	0.86±0.02	0.82±0.01	0.66±0.01**	<0.05
CD8+	%	22.5±0.8	22.1±0.5	22.6±0.4	<0.05
	10^9/l	0.42±0.01	0.40±0.01	0.38±0.01	<0.05
CD22+	%	21.6±0.9	20.6±0.5	21.7±0.6	<0.05
	10^9/l	0.41±0.02	0.37±0.01	0.37±0.01	0.1
CD4/CD8		2.02±0.03	2.05±0.03	1.72±0.02**	<0.01
RBTL with PHA, %		65.5±2.2	63.8±1.3	55.6±1.5*	<0.05

* – P < 0.05 – the probability of divergences regarding the norm.
** – P < 0.01 –the probability of divergences regarding the norm.
*** – P < 0.001 – the probability of divergences regarding the norm.
Column P –degree of probability between indexes of the main group and the comparison group.

Analysis showed that the total population of CD3 + lymphocytes increased only in 1.07 times in patients from the comparison group in relation to the initial value and was 57.5±0.6% after appointed treatment , which is in 1.21 times below normal (P<0.05) and in 1.18 times lower than in patients from the main group (P<0.05). Absolute CD3 + cell count in the peripheral blood of the main group rose during the treatment by an average of 1.44 times and reached the lower limit of the norm, particularly an average of 1.22 ± 0.02 G /l (P>0.05). In patients of the comparison group ,on the other hand, the absolute count of CD3 + lymphocytes during the treatment increased only in 1.13 times and was approximately 0.97±0.01 g/l at the end of therapy, which is in 1.34 times below normal (P<0.05) and in 1.26 times less than in patients of the main group, which were administered with Cycloferon, Erbisol and Amizon.

The CD4+ cell count in the main group was 45.4±0.8% by relative deduction after treatment that is in 1.33 times higher than the initial value and probably did not differ from the norm (P>0.1). During the survey the CD4+ cell count in the comparison group was 38.9±0.7% that was in 1.12 higher comparing to the initial data, but remained below the norm(P<0.05).The absolute count of CD4+ cells in patients with OIGH from the main group was 0.82±0.01 g/l after appointed therapy , which was in 1.52 times higher than the first data and reached the norm (P>0.05). Examination of comparison group, on the other side, showed that the CD4+ cell count was 0.66±0.01 g/l that is in 1.2 times above the original value, but at the same time less than the norm in 1.3 times (P<0.01) and below the corresponding figure in the main group by an average of 1.24 times (P<0.05).

Study revealed that the count of T-suppressors / killers cells with the surface marker CD8 + in patients of both groups did not differ from normal at the end of treatment (P>0.05). The immunoregulatory index CD4/CD8 expressed also a definite tendency to increase in patients from the main group and averaged 2.05 ± 0.03 , which was equal to normal (P>0.1). In comparison group, on the other hand, the CD4+/CD8+ ratio was 1.72±0.02 at the end of therapy, which was less than the norm in 1.17 times (P<0.05). The B cells count with the surface marker CD22+ corresponded to the standard in patients from both groups after appointed therapy (P>0.05).

The RBTL with PHA index that reflects the functional activity of T-lymphocytes increased in patients from the main group in 1.27 times and was 63.8±1.3% after administration of Cycloferon, Erbisol and Amizon (P>0.05). The RBTL with PHA index rose only in 1.1 times and was 55.6±1.5% in patients that were appointed with conventional therapy only . The latter is in 1.18 times below the norm and in 1.15 times lower than the same index in those from the main group (P<0.05).

Analysis of monocyte phagocytosis (PAM) suggests that there was a significant reduction of phagocyic activity of monocytes observed in patients from both groups before therapeutic use (Table 3).

Indeed, there was an indicative decrease of all phagocytic parameters in both groups, especially of the phagocytic index (Table 3).

Table 3. Indexes of PAM in patients with OIGH before treatment (M ± m).

Index of PAM	Norm	Groups of patients with OIGH		P
		main (n=63)	comparison (n=63)	
PI, %	28.6±0.8	17.5±0.9***	17.9±0.6***	<0.05
PN	4.0±0.16	2.7±0.2***	2.8±0.13***	<0.05
IA, %	16.9±0.6	13.1±0.2**	13.5±0.2**	<0.05
II, %	26.5±0.9	14.6±0.7***	14.8±0.1***	<0.05

* – P < 0.05 – the probability of divergences regarding the norm.
** – P < 0.01 –the probability of divergences regarding the norm.
*** – P < 0.001 – the probability of divergences regarding the norm.
Column P –degree of probability between indexes of the main group and the comparison group.

The gained results demonstrate suppression of phagocytic activity of macrophages. The reduction of PAM indexes in the main group and the comparison group was almost the same (P>0.05), which indicates the immunological similarity in all examined. The latter was considered by developing pathogenetical approaches of the therapy for patients with OIGH.

Analysis of macrophage function in patients that were administered with Cycloferon, Erbisol and Amizon revealed that it was a progress in phagocytic activity of monocytes in contrast to the comparison group that exhibit a slight tendency to improvement after appointed conventional therapy (Table 4).

Table 4. Indexes of PAM in patients with OIGH after treatment (M ± m).

Index of PAM	Norm	Groups of patients with OIGH		P
		main (n=63)	comparison (n=63)	
PI, %	28.6±0.8	28.2±0.6	21.9±0.5**	<0.01
PN	4.0±0.16	3.9±0.18	3.5±0.15*	<0.05
IA, %	16.9±0.6	16.6±0.3	14.8±0.2*	<0.05
II, %	26.5±0.9	25.8±0.5	18.6±0.3*	<0.01

* – P < 0.05 – the probability of divergences regarding the norm.
** – P < 0.01 –the probability of divergences regarding the norm.
Column P –degree of probability between indexes of the main group and the comparison group.

A study indicated that PI index increased in 1.61 times approximately and was (28.2±0.6) % at the end of treatment, which corresponded to the norm. The PN, on the other hand, climbed to a baseline by an average of 1.44 times and was 3.9±0.18 which was also within the norm (P>0.05).

Indexes IA and II express a similar tendency to improvement associated with the use of Cycloferon, Erbisol and Amizon and were 16.6 ± 0.3% respectively that is in 1.27 times more than the original value. Moreover, they reached normal limits at the end of treatment (P>0.05).

The PI index showed a rise in 1.22 times in patients from the comparison group appointed with conventional therapy only and at the time of its completion averaged 21.9 ± 0.5%, which was in 1.3 times lower than normal value and in 1.29 times less as the same index in the main group (P<0.01). The PN also rose in 1.25 times in comparison

group and was 3.5±0.15, which, however, was in 1.14 times inferior than normal and in 1.11 times reduced in contrast to the main group (P=0.05). The IA index has experienced a progress as a result of therapy and rose in 1.1 times and was equal to 14.8 ± 0.2%, which, however, was less than the norm by an average of 1.14 times (P<0.05) and below the same indicator in the main group in 1.12 times (P<0.05). The II index was 18.6±0.3% at the end of conventional treatment , which was in 1.26 times higher than the initial data, but below the norm in 1.42 times (P<0.01) and less compering to the main group in 1.39 times (P<0.05).

Conclusion

1. Our study indicates that there were expressed violations of cellular immune related parameters in patients with OIGH that correspond to T-suppressor type of secondary immunodeficiency observed before immunotherapy.
2. Analysis also reveals that the T-cell lymphopenia and imbalance of T cell subpopulations were eliminated in the main group after appointed therapy. There was a rise of T-helpers/inducers (CD4 +) in the peripheral blood, which led to normalization of the immunoregulatory index CD4/CD8 .
3. The suggested therapy that was administered in orthodontical patients with OIGH has caused a normalization of the RBTL with PHA index, which indicated the restoration of functional activity in T lymphocytes.
4. Patients with OIGH benefit from therapeutical use of Cycloferon, Erbisol and Amizon as the results demonstrate a notable improvement of PAM parameters showing an increase in the functional capacity of the macrophage phagocytic system.
5. The developed medical complex provides correction of cellular immunity indexes, restoration of immunological homeostasis in patients with orthodontically induces gingival hyperplasia.

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