ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immunology Biomarkers in Oral Surgery.

Daniela Veleska-Stevkovska[1*], Marija Peeva-Petreska[1], Boris Velickovski[1], Gordana Apostolova[1], Pavlina Aleksova[2], and Filip Koneski[3].

[1] DMD, MSc, PhD, Department of oral surgery and implantology, Faculty of Dentistry in Skopje, Ss Cyril and Methodius University, R. Macedonia

[2] DMD, MSc, PhD Department of restorative dentistry and endodontics, Faculty of Dentistry in Skopje, Ss Cyril and Methodius University, R. Macedonia

[3] DMD, Postgraduate student in dentistry, Resident, Department of oral surgery and implantology, Faculty of Dentistry in Skopje, Ss Cyril and Methodius University, R. Macedonia

ABSTRACT

The initial response during oral surgical interventions is characterized by rapid production and release of various endogenous mediators. The aim of the study was to determine the serum levels of IFN-α and IFN-γ in the perioperative period, as well as the correlation between the serum levels of IFN with sex and age, possible traumatic damage, time interval of the intervention and the possible objective clinical complications throughout the post-operative period. The research was conducted over forty patients with oral surgical interventions divided into two groups (patients with cumulative surgical trauma on soft and hard tissues and patients only with soft tissue surgery). The serum levels of the IFN-α and IFN- γ were registered before the surgical intervention, 24 hours after it, and on the seventh day after it using the ELISA method. Post-operative values of the examined groups showed statistically important increase concerning both groups. With regard to the differences between the groups, there was statistically significant difference in the examined parameter IFN-α. The duration of the intervention, its complexity, the degree of surgical trauma, the body temperatures, as well as the local post-operative complication, were more notable in the first group of patients. The surgical intervention causes an immune and acute phase response, aseptic inflammatory reaction which universally accompanies all kinds of trauma.

Keywords: biomarkers, oral surgery, trauma, immunology, interferons

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The name ,,interferon" (to interfere) origins from his ability to impede viral proliferation. They belong to the group of the cytokines (intracellular signaling) and represent the product of the cells in stimulation with viruses or other foreign substance, yet they do not directly inhibit viral replication. Interferon was discovered by Alick Isaac and Linderman Jean in the year of 1957 [1]. Interferons possess immunoregulatory functions, they inhibit B-limphocyte activation, they activate T cells and increase the destructive ability of NK (natural killer) cells. The division of interferons is executed under the functional characteristics of the protein and by the type of cells which produce that protein. Type 1 interferon is produced by all types of cells stimulated by a viral infection , their primary function is to induce viral resistance into the cells. Type 2 is secreted by the NK cells and T lymphocytes. Their main goal is signaling to the immune system, in order to respond to infectious agents or tumor proliferation. There are three types of inteferons type 1 INF (α and β), type 2 (INF-γ) and type 3 (IL-28,29). IFN-α was previously known as type 1 interferon. It is consisted of 166 aminoacids, with 23 variations of aminoacids, some of them glycosylated. It can be formed from different types of cells, as a respond to recognition of viral and bacterial nuclei acids. It is heterodimer just the same as IRF-9-interferons regulatory factor [2]**.** It has antivirus efect. IFN-α activates surrounded not-infected cells and virus infected as well. As a result of this intracellular activity the cells proteins are formed, which inhibit the further synthesis of viral particals and dismantle the cover of viral RNA. They increase the number of MHC class 1 moleculs which make the viral infected cells more vulnarable to the attack from T cells. IFN-α then activates NK cells (it possess antivirus and antitumor - antiproliferative efect)**.** IFN-γ was previously known as type 2 interferon (Fig.1.). It is a glycoprotein consisted of 143 aminoacids, present in its active form as heterodimer. It is produced by Th1 (T helper) cells and NK cells, in contact with macrophages that fagocyted bacteria. IFN-γ binds to a specific receptor causing signal transduction through the STAT (signal transduction and transcription) molecule. IFN-γ participates in innate and gained immunity. It activates the macrophages at innate immunity and increases their microbicidal function: improves the fusion of fagozom with lysosomes, activates the production of the bacterial nitric oxide and reactive oxygen radicals, induction of antimicrobial lipids, hydrolase induction into the macrophags and activation of 25 (OH) vitamin D3 in the 1,25 (OH) 2 vitamin D3, without the production of the inhibitor on the hydroxylases. IFN-γ stimulated macrophages have increased phagocytic ability and increased ability of destroying intracellular pathogens. Considering innate immunity, it does stimulation of the expression of MHC class I and II molecules and costimulatory molecules of the antigens. It promotes the differentiation of naive T cells into Th1 cells, activating the polymorphonuclears and cytotoxic T cells and increases the cytotoxicity of the NK cells. IFN-γ secreted by Th1 owns crossed regulatory role in controlling the T function and induces reinclusion to IgG. Actually it can inhibit the activities of Th2 via the induction of the IL-12 production by macrophages. This cytokine possesses roles in a number of different types of immune responses such as deferred type of hypersensitivity, inflammation, production of antibodies and viral infections. IFN-γ together with TNF-α are cytokines involved in the management of the macrophages at chronic specific diseases eg. TBC. Also, this interferon may potentiates antiviral and antitumor effects of IFN-α. It possesses the ability of reducing the creation of scarificated tissue (prevents excessive scarification) through blocking the fibroblasts and inhibing the production of TGF-b -molecule of scarification**.**

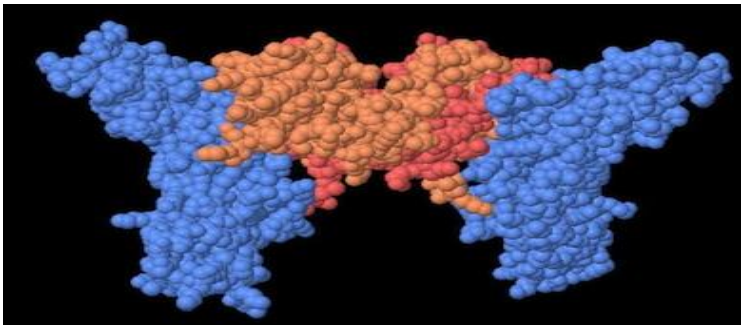

Figure 1. Biomolecular structure of IFN-γ from the protein data bank

The aim of the study was to designate the serum concentrations of IFN-α and IFN-γ in perioperative period; to determine the correlation between the degree of traumatic impairment and the levels of IFN-α and IFN-γ in perioperative period, as well as the correlation between time interval of duration of the intervention and the activity of the examined immune parameter; to determine the possible influence of gender and age

over the immune activity in perioperative period; to determine the correlation between examined parameter IFN-α and IFN-γ and possible clinical complications in postoperative period; to standardize one position for minimization of the traumatic stress, elimination of the cumulative effects of other stressful factors of non-traumatic origin in perioperative period and to determine treatment including immunotherapy in conditions of postoperative immunosuppression.

MATERIALS AND METHODS

In order to accomplish the objectives of the study, 40 patients were encompassed at the Clinic for Oral Surgery at Faculty of Dentistry in Skopje where oral surgical intervention was indicated after the detailed anamnesis, extraoral and intraoral clinical examination and X-ray analysis. Patients signed written consent for performing the examinations. Also the gender and age of the patients were taken into account. According to the type of the oral surgical intervention two groups with 20 patients were formed, patients with diagnosed impacted teeth and patients with indicated soft tissue surgical intervention. In this study patients who did not have clinical signs of infection or neoplasia before the receipt were included and who were not previously treated for at least three months before the scheduled date for intervention. In the individual formular of the patient were registered both quantitative and qualitative characteristics of performed surgical intervention such as: type of intervention, duration of the intervention, the design complexity as well as the degree of surgical trauma to the periosteum and bone tissue (osteotomy). In subjects every presence or absence of objective postoperative complications, were noted down on the first day of the intervention of general (measurement of body temperature) and local character: presence of postoperative pain, edema, hematoma, postoperative infection, trismus and function forest. The seventh postoperative day "suture ex" is performed and it is without clinical features.

Before surgical intervention, 24 hours after it and on the seventh day of the operative intervention in patients were registered the serum levels of IFN-α and IFN-γ. Samples of venous blood were taken in quantities of 10 ml. The blood samples were preserved at 2-6^{o}C to 24h. After that, they were centrifuged and kept at a temperature of -20^{o}C until the moment of the laboratory examination. For the determination of the concentration of cytokines (IL-1, IL-6, TNF-α, IFNα and γ) the ELISA method was used. In determining the level of cytokines we may use various body fluids (urine, serum, plasma, synovial fluids), but as most frequent sample we use plasma and serum. It require 50-200μL serum or plasma. The blood is taken by venipuncture and the serum is separated by centrifuging (5 min. 4000 rpm). If it is necessary to preserve the sample for longer than 1 day, it is held at a temperature of -20^{0}C and if it is necessary the samples to be kept longer than a week, then they are kept at -80^{0} C. The concentration of cytokines in the sample is determined by the ELISA method (enzyme linked immunosorbent assay). Most often used ELISA method uses two types of antibodies. On microtitration plate with 96 wells are bound immobilized antibodies and they serve to bind the examined matter for themselves. After the first rinsing of the wells, the second antibody is added. The second antibody which is detection antibody owns complex enzyme biotin, HRP (horseradih peroksidase) or alkaline phosphatase. After the second rinsing of the wells, amplified solution Amdex is added that represents a specific multifunctional conugate and contains molecules of the HRP and streptavidin. This conugate binds to the cytokine for whom the first-term and second-term antibody are bound. The binding of the antibodies is visible when an enzyme which is bound with the antibody (that detects) and with the amplified solution, reacts with substrate 3,3, 5,5,-tetra-methyl benzidine (TMB). The reaction is stopped by the addition of sulfuric acid. The pigmenting can be read at appropriate wave length with a spectrophotometer or ELISA reader where the intensity of pigmenting is proportional to the examined concentration of the cytokine in the sample. Before start working all chemicals and samples are heat up to a temperature of +20 to 25 ^{0}C. The sensitivity of the method is achieved by adjustment of the volume of the sample, the time of incubation, temperature, as well as the use of different buffers and different pH. In our examinations, the values of the interferons are expressed in pg/ml.

Statistical analysis was manufactured by the statistical program STATISTICA 7.

RESULTS AND DISCUSSION

The group of patients with impactions is made up of 9 (45%) men and 11 (55%) women (Table 1). In Table 2. results are shown concerning the changes of IFN-γ and IFN-α. Table 3.1 and 3.2 expresses the statistical significance of the IFN-γ and IFN-α differences.

ISSN: 0975-8585

Table 1: Distribution by gender

Gender	Number	%
Mail	9	45.0
Female	11	55.0
Total	20	100

Table 2. Changes in interferon: IFN-γ ; IFN-α

Parameter	N	Mean	Confidence - 95.0%	Confidence +95.0%	Min	Max	Std.Dev.
IFN-γ / before	20	1.49	0.54	2.43	0.47	7.65	2.02
IFN-γ / 24h after	20	1.96	1.01	2.92	0.35	7.48	2.05
IFN-γ / 7th day	20	1.46	0.58	2.35	0.44	7.05	1.89
IFN-α / before	20	1.83	1.34	2.32	0.97	4.22	1.05
IFN-α /24h after	20	1.74	1.24	2.23	0.81	4.89	1.06
IFN-α / 7th day	20	1.82	1.34	2.31	0.97	4.23	1.03

Table 3.1. Changes in IFN-γ / differencies

Parameter	N	T	Z	p-level	p	Sig. /N. Sig.
IFN-γ before / IFN-γ 24 hours	20	45.00	2.24	0.025	p<0.05	Sig.
IFN-γ before / IFN-γ 7th day	20	41.5	2.15	0.031	p<0.05	Sig.

Table 3.2. Changes in IFN-α / differencies

Parameter	N	T	Z	p-level	p	Sig. / N. Sig.
IFN-α before / IFN-α 24 hours	20	102.00	0.11	0.911	p>0.05	N.Sig.
IFN-α before / IFN-α 7th day	20	65.00	0.16	0.876	p>0.05	N.Sig.

Christian et al. [3] were investigating the behavior of IFN-γ and IFN-α before and after surgical intervention. They stress the fact that IFN-γ represents a key mediator in the regulation of the immune monocyte ability and is a potent pro-inflammatory mediator. It also helps in overcoming the posttraumatic leukocyte and monocyte paralysis. The subject of the study was to define the potential of IFN-γ as a modifier to monocyte activity before and after surgical trauma. The conclusion of the study is that exogen administration on IFN-γ is effective. De Metz et al. referred to show that IFN-γ has no harmful effects on the metabolite and endocrine system in conditions where the immune system of the host is activated. [4] In the study it is established that IFN-γ is ideal cytokine which improves cell-term immune response in surgical patients without any other additional negative effects on active metabolite and endocrine system. Brand et al. stress that the effects of the surgical intervention, surgical stress and anesthesia compromise the optimal function of the immune system [5]. The authors detect increased levels of IFN–γ by surgical intervention.

Correlations (only statistically significant correlations are given)

The examined relationship between the duration of the intervention and IFN-γ (before intervention), for r = 0:53 (p <0.05) is a medium strong and significant, i.e. the increase of the time of intervention is followed by increasing the values of IFN- γ (before).

When analyzing the results of the relationship between the degree of surgical trauma and analyzed parameters, no significant relations are registered (p <0.05). A number of authors are investigating the mechanisms of the tissue impairment, stress-conditioned tissue impairment and the mediators in tissue impairment that bring to the activation of the non-specific and specific immunity. Watson T.[6] as well as Wagner [7] revise the four processes that occurs during the damage of the soft tissues: bleeding, inflammation, proliferation and remodeling. In the process of inflammation there are two events as follows:

vascular and cellular cascade. After the initial vasoconstriction, vasodilation comes with changes in the caliber of blood vessels, changes in the wall of the blood vessels and their permeability as well as changes in blood flow through them. The reason for these changes in the blood vessels are chemical substances, histamine, prostaglandins, complement cascade, leukotrienes, serotonin (5-HT) and the axon reflexes. In relation to the cellular events during the inflammatory response, the authors stress the early migration of PMN polymorphonuclear neutrophils) as well as (monocytes, lymphocytes, eosinophils, basophils and a small number of red blood cells). The main chemical mediators responsible for chemotaxis are the components of the complement system, the factors released by PMN , peptides from fat cells and damaged tissue, PDGF (platered derived growth factor) released from the injured platelets, leukotrienes (free of leukocytes, fat cells and macrophages) and lymphokines (free of PMN). Sota Omoigui pays attention to the existence of complex interactions of inflammatory mediators that are exempt by traumatic tissue impairment and describes the details of different paths of their creation (bradykinin, phospholipase A2 enzyme, arachidonic acid, substance P, MMP-9 which includes the collagenase and stromelysin)[8]. He clearly precize the difference of the effects on the immune system in different types of tissue as well as the quantitative level of the liberation of the mediators during inflamation at different types of tissue (bone tissue contains the larger number of mediators during the tissue impairment than soft tissue). Wichmann and Ayala are investigating the impact of the isolated tissue damages at soft tissues or bone tissues as well as combined tissue damages (soft tissue and bone tissue) on the immune system, and they come to the conclusion that at combined tissue impairments there exist significant depression of cytokine production compared to isolated tissue impairment[9]. Heating of the bone tissue in oral surgical interventions according to Sternfeld and Ogle qualitatively change the monocyte-macrophage function even it quanititatively and qualitatively influence the hematopoiesis.[10] These cells produce less acute phase proteins (APPs), C3 and transferrin, but have greater cytotoxicity measured according to the liberation of 1-lactate dehydrogenase. In our study the group of patients with impactions, respondents differ between themselves only with the number of osteotomised surfaces, which seems to give discrete impact on systemic analyzed parameters (weak correlation). When considering locally produced mediators, the organism according to its individual abilities, manages to sustain them localy (to keep them localy). This represents a good outcome in postoperative process in examined sample.

Table 4. Duration of the intervention/analyzed parameters

Parameter	Pearson / r	P	Sig. / N.Sig.
IFN-γ / before	0.53	p<0.05	Sig.
IFN-γ / 24 hours	0.19	p>0.05	N.Sig.
IFN-γ / 7th day	0.50	p<0.05	Sig.
IFN-α / before	0.05	p>0.05	N.Sig.
IFN-α /24 hours	0.03	p>0.05	N.Sig.
IFN-α / 7th day	0.06	p>0.05	N.Sig.

Table 5. Trismus/analyzed parameters

Parameter	Spearman / R	P	Sig. / N.Sig.
IFN-γ / before	-0.47	p<0.05	Sig.
IFN-γ / 24 hours	-0.25	p>0.05	N.Sig.
IFN-α / before	-0.09	p>0.05	N.Sig.
IFN-α / 24 hours	0.22	p>0.05	N.Sig.

Table 5. presents the results of the examined relationship between trismus and analyzed parameters. This relationship between the changes of trismus and the value of IFN-γ (before intervention) for R = -0.47 (p <0.05) shows a medium strong significant negative correlation, where the values of IFN-γ before intervention decrease, and the values of the trismus increase. This data refer to the fact that as much the values of interferon are lower considering the individua (preoperative), that much the probability of the occurrence of trismus increases (due to the local complications: infection, trauma).

In Table 6. and Table 7. the results are presented concerning the differences between the analyzed parameters, between the groups.

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Table 6. Differences considering the analyzed parameters between the groups.

Parameter	Rank sum / Group 1	Rank sum / Group 2	Z / t	p-level	p	Sig. / N. Sig.
IFN-γ / before	339.50	480.50	-1.91	0.057	p>0.05	N.Sig.
IFN-γ / 24 hours	411.00	403.00	0.19	0.849	p>0.05	N.Sig.
IFN-γ / 7th day	347.00	473.00	-1.70	0.088	p>0.05	N.Sig.
IFN-α / before	347.00	473.00	-1.70	0.088	p>0.05	N.Sig.
IFN-α /24 hours	330.00	490.00	**-2.16**	0.030	p<0.05	**Sig.**
IFN-α / 7th day	342.00	478.00	-1.84	0.066	p>0.05	N.Sig.
Duration of the intervention	601.50	218.50	**5.18**	0.0000	p<0.001	**Sig.**
Complexity of the intervention	570.00	250.00	**4.33**	0.0000	p<0.001	**Sig.**
Degree of surgical trauma	210.00	610.00	**-5.41**	0.0000	p<0.001	**Sig.**
Body temperature	580.50	239.50	**4.61**	0.0000	p<0.001	**Sig.**

Table 7. Differences concerning local complications

Parameter	Rank sum / Group 1	Rank sum / Group 2	Z / t	p-level	P	Sig. / N. Sig.
Pain	562.00	258.00	**4.11**	0.0000	p<0.001	**Sig.**
Edema	556.50	263.50	**3.96**	0.0001	p<0.001	**Sig.**
Hematoma	400.00	420.00	-0.27	0.787	p>0.05	N.Sig.
Trismus	550.00	270.00	**3.79**	0.0002	p<0.001	**Sig.**
Function disorder	550.00	270.00	3.79	0.0002	p<0.001	Sig.
Regional lymphadenitis	520.00	300.00	2.98	0.003	p<0.01	Sig.
Infection	410.00	410.00	0.00	1.00	p>0.05	N.Sig.

Significant differences are registered between the following parameters:

- for Z = -2.16 and p <0.05, 24 hours from operational intervention in the group of patients with soft tissue surgery (group 2), the value of IFN-α is significantly higher than in the group of patients with impactions (group 1). Namely, the higher the degree of surgical trauma, the greater is the probability of postoperative decrease of the values of IFN-α e.g. there is a greater probability of immunosuppression, or inadequate response to the organism.
- for Z = 5:18 and p <0.001, duration of the intervention in the group of patients with impactions (group 1) is significantly longer than in the group of patients with soft tissue surgery (group 2).
- for Z = 4.33 and p <0.001, complexity of the intervention in the group of patients with impactions (group 1) is significantly higher than in the group of patients with soft tissue surgery (group 2).
- for Z = -5.41 and p <0.001, the degree of surgical trauma in the group of patients with impactions (group 1) significantly dominates compared to patients with soft tissue surgery (group 2). That is how much the degree of surgical trauma is higher (soft tissue, periosteum, bone tissue) that much the greater is the probability for reduced response to the organism e.g. immunosuppression.
- for Z = 4.61 and p <0.001, body temperature by intervention in the group of patients with impaction (group 1) is significantly higher than in patients with soft tissue surgery (group 2).
- for Z = 4:11 and p <0.001, the pain after the intervention in the group of patients with impaction (group 1) is significantly present than in patients with soft tissue surgery (group 2).
- for Z = 3.96 and p <0.001, after the intervention edema was significantly registered in the group of patients with impaction (group 1) than in patients with soft tissue surgery (group 2).
- for Z = 3.79 and p <0.001 there is a significant difference considering trismus, namely after the intervention trismus is only registered in the group of patients with impaction (group 1), in patients with soft tissue surgery (group 2) is not registered.
- for Z = 3.79 and p <0.001 there is a significant difference considering the disorder of the function, namely after the intervention, disorder is only registered in the group of patients with impaction (group 1), in patients with soft tissue surgery (group 2) is not registered.
- for Z = 2.98 and p <0:01 there is a significant difference concerning the presence of regional lymphadenitis, namely after the intervention, the regional lymphadenitis was only registered in the

group of patients with impaction (group 1), in patients with soft tissue surgery (group 2) is not registered.

In the other analyzed parameters for $p>0.05$, no significant difference was determined.

CONCLUSIONS

- Surgical intervention causes immune and acute phase response, the aseptic inflammatory reaction, which universally accompanies all types of trauma.
- Rapid increase in the levels of interferons postoperatively, demonstrate and prove high sensitivity. From the obtained results of the examined parameter, we concluded that it is justified to use the diagnostic tests for determining the levels of interferons as landmark for the degree of postoperative inflammation and they represent effective prognostic tool. Knowledge of the interferon levels may give us insights to intracellular changes (intracellular milieu) as well as the directions in further treatment.
- According to our examination and according to a number of studies the thesis is confirmed that the greater is the degree of surgical trauma (soft tissue, periosteum, bone tissue) the greater is the probability for unadequate respond to the organism e.g. immunosuppression. In the group of patients with impactions, respondents differ between themselves only with the number of osteotomised surfaces which seems to give discrete impact on systemic analyzed parameters (weak correlation). According to its individual abilities, the organism manages to sustain the locally produced mediators (to keep them locally). This represents a good outcome in postoperative process at examined sample.
- There is a medium strong significant negative correlation between the values of IFN-γ preoperative and the manifestation of the postoperative trismus. This data refer to the fact that as lower the values of the interferon (preoperative), the higher is the probability of the occurrence of trismus as local postoperative complication.
- The minimum pain, faster ,,recovery" and shorter hospital process are the benefits of minimally invasive surgery. Here comes the recommendation for the selection of the methods of the modern minimally invasive surgery before the methods of conventional surgery, in all those cases where it is indicated.
- In accordance with the previously reported conclusions we can see the importance of minimizing the traumatic stress underway to oralsurgical interventions, by favoring non-traumatic approach, minimizing the pressure and vibrations during the intervention, reducing the temperature which increases during osteotomy (using systems with continuous internal and external cooling, reducing the number of spins in minutes of the instruments), using burs from high quality materials and with sufficient rigor.
- With equal importance is the elimination of stress factors of non-traumatic origin through the use of premedication (anxiolytics, analgesics) as well as psychological techniques and strategies for the reduction of stress (stress reduction strategies).
- Antibiotic and anti-inflammatory prophylaxis is recommended in complicated and long-lasting interventions.

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