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NEW ALGORITHM OF INJECTION PRP - THERAPY IN PARODONTOLOGY

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Abstract

A step-by-step algorithm of a dentist's actions directed to platelet autoplasma obtaining for the further therapy of periodontal diseases with platelet-rich plasma (PRP) has been developed on the basis of laboratory and clinical data. The method consists of preclinical (laboratory) and clinical stages. On the basis of laboratory data and coefficient of platelet concentration the authors developed mathematical formulas which allow to calculated an individual volume of autoplasma for a single injection, its total volume, the number of test-tubes necessary as well identify indications and contraindications for the method. The use of the method developed will improve the quality of the patients with multisystem periodontal disease treatment due to optimal stimulation of periodontal tissues regeneration.

Key words: generalized periodontitis, platelet-rich plasma, platelet autoplasma, platelets concentrating ability.

Among the diseases of periodontal tissues generalized periodontitis (GP), occupies leading positions with its prevalence of 86-95% among the adult population of Ukraine. In this regard, effectivization of its complex therapy, aimed to stabilize the pathological process

in the periodontal tissues and achievement of a long-term remission is an urgent task [1, 3, 7, 10].

Injection of platelet-enriched plasma (PRP-therapy) is considered a modern, safe and affordable method of treatment, due to which it is widely used in various fields of medicine. The method under discussion is based on regenerative technologies, aimed at stimulation of reparative processes in various tissues of the body [6]. The possibility of obtaining such an effect, first of all, is due to the presence in the platelets of a large number of various growth factors that are released under their breakdown [2, 11, 12]. The very concept of "Platelet-Rich Plasma" implies that the plasma obtained by centrifugation of the patient's autologous venous blood should contain a platelet count that does not just significantly exceed their normal (physiological) level, but should be in the range of 500,000-1,000,000 / μL (500,000,000 - 1,000,000,000 / ml for the provision of positive therapeutical effect [13, 14, 15].

The works of many authors present perspectives of PRP injections in the complex treatment of generalized diseases of periodontal tissues of different genesis [2, 12] before injection of platelet autoplasma.

However, in the literature available, we did not meet any indications to the necessity of individual oscillations of platelets number control in a patient's native blood before injection of autoplasma, as well as taking into account the concentration ability of platelets after centrifugation. Meanwhile, the results of our studies [8, 9] show that it is the level of these indexes that determines the effect of stimulation of regenerative processes in PRP-therapy both at the stage of treatment and at the stage of prevention of generalized periodontal diseases. In addition, we found [4] that the amount of platelets that enters the tissue with the injection of autoplasma can be regulated by the volume of administration of the drug.

In this regard, count of thrombocytes in the native blood, estimation of their concentration ability to obtain PRP, determination of its optimal volume for further injection, should be taken into account when the method under consideration used in combination of preventive and therapeutic interventions in generalized periodontal diseases.

The objective: to develop an algorithm of PRP-injection therapy in parodontology, based on the preparation of platelets-rich autoplasma medication in an amount providing the highest effect of regenerative processes stimulation.

Materials and methods. The samples of venous blood were collected in tubes containing different anticoagulants (Na-heparin, 3.2% p-or sodium citrate). There were 40 patients (mean age - 44.9 ± 13.26 years) under examination. The total number of tubes - 120 pieces: 40 certified tubes with Na-heparin; 40 tubes with 3.2% sodium citrate solution; 40

tubes with EDTA anticoagulant. Blood sampling for determination of platelets number was performed from the ulnar vein in the morning on an empty stomach by the TERUMO system (Belgium).

To obtain heparin and citrate platelet autoplasma 80 venous blood samples were centrifuged (a laboratory centrifuge CM-6M-Elmi, Latvia). Then the number of platelets in native blood, in heparin and citrate plasma after centrifugation was determined on the hematological analyzer BC-3000 Mindray (China). The principle of determination is Coulter's conductometric (impedance) method.

The statistical analysis of the results was carried out with the Microsoft Excel software packages.

Results. It has been established that not every plasma obtained by centrifugation with different anticoagulants comprises platelets in an amount necessary to obtain a pronounced effect of stimulation of regenerative processes. This completely changes the methodology of performing the known method of injection PRP-therapy.

First of all, the necessity of the pre-laboratory stage, i. e. determination of the platelets number in the native blood, calculation of their concentration ability, determination of the optimal number of platelets in the individually calculated volume per intratissual injection with the most pronounced effect of regenerative processes stimulation at the complex of preventive or therapeutic measures delivery is obvious.

Determination of the platelets number in native blood and platelet concentration coefficient (PCC) allows to predict the possibility of the expected effect of stimulation obtain, and initially identify contraindications to the use of this method. So, if the platelets content in native blood is significantly reduced, this indicates that the patient has thrombocytopenia and, therefore, to obtain pronounced, stimulating reparative processes the effect is impossible.

Evaluation of platelets concentration ability is quite easy to make with the help of the mathematical indicator - PCC, which, on the one hand, is simple enough for the doctor's perception, and on the other, has an extraordinary information value, since it allows easily estimate the individual saturation of a patient’s plasma with platelets, after centrifugation in test tubes with different anticoagulants (different production), which makes the indicator mentioned universal.

The PCC is determined by the following formula:

PCC = PCAC/PCNB, *where:*

- PCAC is platelets concentration after centrifugation;
- PCNB is platelets concentration in native blood

Calculation of PCC after centrifugation allows us to evaluate immediately the potential effectiveness of PRP-therapy. The value of PCC <1 indicates that a "so-called" platelets poor plasma (PPP) was obtained, where the number of platelets after centrifugation is less than in native blood. So, it is quite obvious that the expected regenerative result will not be obtained after PRP-therapy and this method is not indicated for treatment; PCC = 1 indicates that the number of platelets after centrifugation is not different from their content in native blood, and therefore, to expect a pronounced regenerative result in this situation is in vain; PCC> 1-4 indicates the production of plasma enriched with platelets, and its use for injection PRP-therapy it is possible to obtain a pronounced regenerative effect.

Then one should take into account the individual volume of plasma required for one administration. Here in addition to PCC, it is necessary to know what is the number of platelets injected (NPI) is optimal for the stable therapeutic effect to obtain. It is known that the optimal concentration of platelets, which provides a positive therapeutic effect in PRP, is 500 000-1000 000 / μL (500000000 - 1000 000 000 / ml) [1, 2]. Meanwhile, we know that the volume of plasma for injection in the area of the transitional fold in patients of the dental profile is limited by the anatomical features of this zone and on average is 0.3-0.5 ml [4]. It is obvious that in order to realize the task posed - the effect of the most pronounced stimulation of regenerative processes in the periodontium, the number of areas for injection should be increased. This can be solved only by additional plasma injection in a volume of up to 0.5 ml per injection in the apex projection area of every 1-2 teeth of the upper and lower jaws using the circular technique developed by us [5].

Then we should determine the minimum number of platelets (MNP), which at a single injection to a patient, will result in a high therapeutic effect. To answer this question, we proposed the formula:

MNP = 500 000 000 NPI / ml × 0.3 ml = 150 000 000 NPI

So, the number of platelets necessary should be in the limits 150.000.000 NPI.

However, taking into account that the platelet content in a human's native blood surges, the concentration capacity of platelets after centrifugation is variable index and MNP per a single injection should be at least 150 000 000 it is obvious that the volume of plasma for a single injection should be calculated for each patient individually.

Therefore, in order to calculate the total volume of platelet autoplasm (V, ml) required for one PRP therapy procedure, the following formula was proposed: V (ml) = v (ml) × n, where: v (ml) is the individual volume of plasma per one injection; n is the multiplicity of plasma injections.

Proceeding from the fact that in one tube after centrifugation there is an average 3 ml (from 2 to 4 ml) of autoplasm, the doctor should perform an additional calculation in order to determine the number of tubes necessary for one treatment procedure:

Nt = V (ml) / Vav (ml), where: Nt-number of test tubes; Vav (ml) = 3 ml - the average volume of autoplasma in one tube; V is the total volume of platelet autoplasm necessary for one treatment procedure.

Thus, our researches have shown the need to review the known methods of injecting PRP-therapy, and it resulted in of which a new technique development. The latter consists of two stages: preclinical (laboratory) and directly clinical. The algorithm is based on the task of increasing the efficiency of stimulation of regenerative processes with a therapeutic or prophylactic purpose due to the preliminary determination of the number of platelets in the native blood and the intake of an optimal amount of platelets in the individually calculated volume per injection.

At the first stage, this task is realized with the additional use of the mathematical index - the coefficient of platelet concentration (CPC), which is quite simple for the doctor's perception and allows easily evaluate the individual plasma saturation of platelets after centrifugation in tubes of different production, which makes it universal.

I stage - preclinical (laboratory).

• CBC, which allows to determine the number of platelets in native blood and identify the indications for the technique;

• Centrifugation of tubes with native blood.

- get autoplasma and set the number of platelets in it.

Stage II - clinical.

• determine the PCC (PCC = PCAC / PCNB)

• determine the individual volume of plasma - V (ml) necessary for one injection

(V = MNP (PLT) / PCNB (PLT / ml) × PCC);

• determine the total volume of platelet autoplasma - V (ml), necessary for one procedure of PRP- therapy

(V (ml) = v (ml) × n);

• determine the number of tubes required for one treatment procedure - Nt = V (ml) / Vav (ml);

• to inject the drug on the transitional fold, using a circular technique - the most effective one in the generalized periodontal diseases.

The advantage of the algorithm developed is that the dentist can correctly calculate the individual volume of plasma for a single injection, the total volume of plasma that should be injected per in a single procedure, and determine the number of tubes necessary. The optimal amount of platelets, which the doctor will introduce into the periodontal tissue, thanks to the individually selected volume of administration, will provide a stable therapeutic effect due to the pronounced stimulation of the regeneration processes in the periodontal tissues.

By example of five patients with generalized periodontitis of I – II degree (mean depth of gingival pocket 3,8 – 4.5 mm) we suggest to have a look at practical realization of the method developed. All the patients were treated at our department. After completion of the initial periodontal treatment, the patients were offered PRP-therapy and informed consent was received. Then they were sent to the laboratory of A. Shalimov National Institute of Surgery and Transplantology (head of the department of laboratory diagnostics Deev VA, cand. med scien.). The patients' venous blood was obtained, general blood test was done and the number of platelets determined. The blood obtained was centrifuged in test-tubes with different anticoagulants and the number of platelets and their concentration capacity was determined.

The number of platelets in native blood and plasma enriched with platelets was determined on the hematological analyzer BC-3000 Mindray (China). The principle of determination is Coulter's conductometric (impedance) method.

At the 2nd stage, based on the laboratory data, the calculations of PCC and individual volume of autoplasma by the formulas proposed were made. The calculations' results are presented in Table 1.

Table 1

Determination of indications and optimal volume of platelet-enriched autoplasma at PRP-therapy

N	Sex	PCNB	PCC	v, ml	V, ml (n=16)	Nt
1	M	171000000	1.61	0.54	8.664	3
2	M	55000000	3.11	0.87	13.92	5
3	F	258000000	1.37	0.42	6.72	2
4	M	210000000	3.04	0.23	3.68	1
5	F	237000000	0.6	1.05	16.8	6

The data presented in Table suggests that PRP-therapy is not indicated to the patients 2 and 5. Patient's 2 native blood has a significantly reduced number of platelets which indicates the presence of thrombocytopenia, and patient's 5 PCC is significantly reduced (PCC <1). Thus, in both cases, the PRP-therapy expected regenerative effect can not be obtained.

Patients' 1, 3 and 4 platelets concentration ability was > 1, which is an indication for PRP - therapy. However, Patient's 1 initial content of platelets in the native blood is slightly lower than the reference intervals (180-400 x 10^9 / l), and in patient 3 with a normal blood platelet count, a certain decrease in their concentration ability was found. Individual calculations done indicate the need, firstly, to increase the volume of plasma for one injection to 0.54 ml and 0.42 ml, respectively, and secondly, to increase the number of tubes with anticoagulants required for one treatment procedure to 3 test tubes for patient 1 and up to 2 tubes in patient 2. The calculations carried out for patient 4 showed that he had the best conditions for PRP therapy. So, with a normal initial platelet count in blood, this patient has a high platelet concentration capacity (PCC = 3.04), which allows to reduce the volume of single plasma injection to 0.23 ml, and for which only 1 tube is enough. So the cost of 1 PRP – therapy procedure depends on the number of test tubes necessary. Thus, PCC > 2 - 4 with the blood platelet content within the reference values allows to minimize the costs for 1 PRP - therapy procedure without decrease of the expected therapeutic effect, which is of great importance both for the doctor and for the patient's motivation.

Conclusions. Thus, based on the laboratory and clinical data obtained, a step-by-step algorithm of a dentist's actions of platelet autoplasma for PRP - therapy getting consists of two stages: preclinical (laboratory) and clinical.

Implementation of mathematical formulas developed based on laboratory diagnosis data and definition of PCC will allow the dentist: correctly calculate the individual volume of autoplasma for a single injection; determine the total volume of plasma with the exact number of tubes required for a single procedure; to reveal indications and contraindications for PRP - therapy.

The introduction of the method of injection PRP-therapy in periodontology will certainly contribute to improving the quality of treatment for patients with generalized periodontal diseases by optimizing the approach to stimulation of regenerative processes in periodontal tissues, which are of great importance at the stage of conservative treatment.

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