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Адрес редакции:
100008, Республика Казахстан,
г. Караганда, ул. Гоголя, 40, к. 130
Тел. +7 (7212) 50-39-30 (1286)
Моб. +7 701-366-14-74
Факс: +7 (7212) 51-89-31
E-mail: Serbo@gmu.kz

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A. R. Koshkinbayeva[1]*, A. V. Ogizbayeva[2], O. A. Ponamareva[3], B. K. Omarkulov[1], Ya. A. Yutskovskaya[4]

EFFICIENCY AND SAFETY OF AUTOLOGOUS PLATELET-RICH PLASMA

[1]Department of Family medicine, NJSC «Karaganda Medical University» (100008, Republic of Kazakhstan, Karaganda, Gogolya St., 40; e-mail: shar_surgeon89@mai.ru)
[2]Department of Emergency Medical Care, Anesthesiology, and Resuscitation, NJSC «Karaganda Medical University» (100008, Republic of Kazakhstan, Karaganda, Gogolya St., 40; e-mail: eleusizova.a@kgmu.kz)
[3]Department of Biomedicine, NJSC «Karaganda Medical University» (100008, Republic of Kazakhstan, Karaganda, Gogolya St., 40; e-mail: Ponamareva@qmu.kz)
[4]LLC «Clinic of Professor Yutskovskaya» (129626, Russian Federation, Moscow, inner-city municipal district Alekseevsky, Mira Avenue, 102, building 26, room 8N/2; e-mail: ryazanova_lora@mail.ru)

***Alina Robertovna Koshkinbayeva** – PhD student, Department of Family medicine, NJSC «Karaganda Medical University»; 100008, Republic of Kazakhstan, Karaganda, Gogolya St., 40; e-mail: shar_surgeon89@mai.ru)

The purpose of this literature review is to provide relevant research results on the efficiency and validity of the platelet-rich plasma in various fields of medicine as well as to cover some unresolved issues and problems in this area. If one searches the keywords such as «platelet-rich plasma», «safety», «effects» in the *Scopus* and *PubMed* databases, 590 publications will be found over the past 5 years, most of which are in the field of orthopedics, gynecology and dermatology.

Autologous plasma enriched with platelets is widely used today in various fields of medicine. The platelet-rich plasma is so widely used because it releases numerous chemokines, cytokines, and growth factors that stimulate angiogenesis, extracellular matrix remodeling, and cell differentiation and proliferation. Numerous studies have shown positive local effects of the platelet-rich plasma: earlier vascularization, epithelialization, remodeling and tissue regeneration. However, current studies on the clinical efficiency of the platelet-rich plasma are not conclusive and homogeneous. The heterogeneity of the platelet-rich plasma specimens (different preparation methods, various concentrations of platelets and growth factors, the influence of age, sex, concomitant pathology of the patient) complicates the interpretation of the existing literature and limits the ability to give definitive recommendations on the clinical efficiency of the platelet-rich plasma. Presently, there is no comprehensive standard algorithm for the platelet-rich plasma preparation as well as no definite criteria for establishing indications for this procedure. The following issues remain unresolved: finding an effective method to prepare the platelet-rich plasma, determining the optimal concentration of platelets and growth factors, having insufficient knowledge about all molecular mechanisms of the platelet-rich plasma to promote tissue regeneration and systemic reactions to the introduction of the platelet-rich plasma. Further research is required to address all the issues raised.

Key words: platelet-rich plasma; efficiency; safety; growth factors; regeneration

INTRODUCTION

Autologous plasma enriched with platelets (platelet-rich plasma, PRP) is widely used today in various fields of medicine: traumatology and orthopedics, ophthalmology, gynecology, plastic and reconstructive surgery, aesthetic medicine, trichology, dermatology. It is also used in case of any burns and trophic ulcers, etc. [1]. PRP gained its widespread use because it released numerous chemokines, cytokines, and growth factors stored in platelet alpha granules and was able to improve tissue regeneration. The processes affected by these molecules include angiogenesis, extracellular matrix remodeling, and stem cell differentiation and proliferation [9, 14].

However, the current clinical efficacy studies on PRP are not conclusive and homogeneous. One of the main reasons is in using different PRP preparations containing different platelet counts and growth factors, causing reactions of various types, which cannot be compared with each other [2]. In numerous clinical studies, the PRP preparation protocols' reports have been highly inconsistent, and some have failed to indicate the PRP preparation methods required for a reproducibility protocol. Additionally, there is no clear consensus on the indications for treatment in various pathologies, which makes it difficult to compare the efficiency and safety of PRP [10]. Thus, it is required to standardize the PRP preparation algorithms, PRP administration procedure algorithms as well as to determine the criteria for admission of patients with different pathologies to this procedure.

The aim of this literature review is to provide relevant research results on the efficiency and validity of the PRP's use in various fields of medicine, as well as to cover the unresolved issues and problems in this area. The «platelet-rich plasma», «safety», «effects» were used as the keywords in the Scopus' and PubMed's databases to locate 590 publications over the past 5 years, most of which were in the field of orthopedics, gynecology and dermatology.

WHY IS AUTOLOGOUS PLATELET-RICH PLASMA USED?

The main function of platelets is to prevent acute blood loss and restore the vascular walls and adjacent tissues after injury. Platelets are activated by contact with collagen entering the bloodstream after endothelial damage. Platelets secrete mediators and cytokines from the cytoplasmic pool and release the contents of their α granules. This secretion is mostly intense in the first hour, and even later on, platelets continue to synthesize cytokines and growth factors for at least another 7 days [2].

It was discovered in 1978 that a 10% serum could significantly facilitate smooth muscle cell proliferation in the in-vitro experiments, but this effect of stimulating cell proliferation disappeared after replacement with platelet-depleted serum [30]. In the same year, the platelet-derived growth factor (PDGF) was found in platelet alpha granules, and in the next 20 years it was discovered that platelets also contained other growth factors: transforming β growth factor (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), etc. [36].

The growth factors secreted by platelets have a paracrine effect on different types of cells: myocytes, tendon cells, mesenchymal stem cells of various origins, chondrocytes, osteoblasts, fibroblasts and endothelial cells [2]. This concurrently stimulates cell proliferation, angiogenesis and cell migration, which lead to tissue regeneration. The biologically active substances secreted by platelets provide a suitable micro-environment for tissue repair, thereby affecting the wound healing process [8]. There are studies to confirm that platelets have properties related to their anti-inflammatory and analgesic effects, and can also secrete antimicrobial peptides [2]. In addition, it has been suggested that platelets can activate peripheral blood mononuclear cells (PBMC), which then secrete interleukin-10 (IL-10), an anti-inflammatory cytokine, which is also involved in tissue regeneration [29].

With all these properties taken into consideration, the use of PRP is increasingly gaining its popularity in various fields of medicine.

SUBSTANCES RELEASED BY PLATELETS INTO PRP

Platelet alpha granules secrete numerous vital factors for regeneration: transforming growth factors (TGF-β1, TGFβ2), platelet growth factors (PDGFAA, PDGFBB, PDGF-AB), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-I), epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) [18] (Table 1).

These factors in platelets, especially TGF-β, regulate cell proliferation, extracellular matrix synthesis, angiogenesis, and local immune responses. In addition, TGF-β induces the differentiation of keratocytes into myofibroblasts, which is necessary for the healing process of severe stromal wounds [1].

It has been suggested that the potential ergogenic effect of PRP is due to the influence of insulin-like growth factor-1 (IGF-1), which is involved in muscle proliferation, differentiation and regeneration. After tissue damage by mechanical stimuli, the IGF-1Ec isoform in muscles, tendons, and bones causes cellular proliferation [7, 37].

In addition, all growth factors act synergistically, increasing the local concentration of mesenchymal stem cells, macrophages, and fibroblasts that facilitate angiogenesis, fibroplasia, and reepithelization, which ultimately induces tissue regeneration [13].

Furthermore, the molecules in PRP, such as HGF, have an anti-inflammatory effect. The platelet granules also contain calcium ions, serotonin, histamine, dopamine, adenosine diphosphate (ADP), and adenosine triphosphate (ATP), which are essential for tissue homeostasis. The released serotonin also has an analgesic effect. In addition to these growth factors, platelets contain other substances such as fibronectin and vitronectin, which are cell adhesion molecules playing an important role in the connective tissue matrix [10, 17].

THE PRP APPLICATION IN VARIOUS FIELDS OF MEDICINE

The first studies on the development and application of PRP were initiated in the early 1970s in the field of hematology [7]. Since the 1990s, PRP has been used for a variety of purposes and in various fields of medicine. In 1998, PRP was first used to restore the defects of the lower jaw. It was found that PRP could significantly reduce the process of osteogenic repair. Since then, PRP has gradually been used in orthopedics to improve bone fusion and fracture repair, as well as to accelerate the recovery of soft tissues in acute and chronic tendon injuries [36]. To this day, a majority of the research on PRP has been done in the field of orthopedics.

A systematic review of the effects of PRP on ligament and tendon injury found a positive molecular effect of PRP on tissue healing and remodeling. The use of PRP has been proved to stimulate the proliferation and migration of fibroblasts and tenocytes to the affected area and enhance the activation of the expression of the type I collagen gene, which leads to healing of tissues with better biomechanical properties [4]. There was also a significantly early increase in the rate of ligamentization and vascularization along with an early reduction in inflammation [28]. As a result of another systematic review and meta-analysis, the authors concluded that PRP lead to a reduction in pain after its administration in those patients treated with lateral epicondylitis and osteoarthritis of the knee [10].

In ophthalmology, with corneal injuries, the administered PRP induced the rapid formation of neovascularization and accelerated stromal remodeling by inducing the myoblasts formation in the deep tissues of the cornea [6].

Table 1 – Effects of the main growth factors in platelet-rich plasma

Growth factor	Effect
Platelet derived growth factor, PDGF	Mitogenesis, angiogenesis, regulation of other cells and growth factors (stimulation of fibroblasts and osteoblasts, induction of cell differentiation, catalyzing the influence of other growth factors on macrophages), regulating the secretion of collagenase and collagen synthesis, stimulating the chemotaxis of macrophages and neutrophils.
Transforming growth factor- beta, TGF-β	It stimulates undifferentiated proliferation of mesenchymal cells, regulates endothelial, fibroblastic and osteoblastic mitogenesis, collagen synthesis and collagenase secretion, mitogenic effects of other growth factors, stimulates endothelial chemotaxis and angiogenesis, bone matrix formation, inhibition of bone resorption.
Insulin-like growth factor, IFG-1	Chemotaxis of fibroblasts, proliferation and differentiation of osteoblasts, formation of bone matrix, growth and restoration of skeletal muscles.
Epidermal growth factor, EGF	Proliferation and differentiation of epithelial cells, keratinocytes, fibroblasts, stimulation of mitogenesis of endothelial cells
Vascular endothelial growth factor, VEGF	Angiogenesis of endotheliocytes, stimulates mitogenesis of endothelial cells, chemotaxis of macrophages and granulocytes, vasodilation.
Basic fibroblast growth factor, bFGF	Stimulates the differentiation of bone marrow stem cells, enhancement of calcium deposition, stimulation of growth and differentiation of chondrocytes and osteoblasts, mitogenesis for mesenchymal cells, chondrocytes and osteoblasts.
Hepatocyte growth factor, HGF	Regulates the growth and mobility of epithelial/endothelial cells, supporting epithelial repair and neovascularization during wound healing.

PRP has been used in skin plastic surgery for burns, injuries and removed tumor, while patients experienced a decrease in the severity of pain and itching, and epithelialization was much faster than in the control group [1].

In gynecology, the effect of PRP-therapy in endometrial inflammation, ovarian diseases, thin endometrium, recurrent implantation failure has been investigated [18]. The PRP treatment has been reported to lead to an increase in endometrial thickness (confirmed by ultrasound), an increased incidence of pregnancy (clinically confirmed), and an increased incidence of live birth [21]. Some researchers have suggested that PRP is able to stimulate the migration and proliferation of endometrial mesenchymal stem cells, which subsequently differentiate into endometrial cells.

In sports medicine, PRP is of interest as an accelerator of muscle injury healing and therefore, may allow a patient to quickly resume daily sports-related activities. Many authors report that the growth factors and the fibrin matrix are critical to the process of muscle repair and regeneration, promoting myogenesis, angiogenesis, and fibrogenesis [7, 19].

Moreover, PRP is presently in great demand in aesthetic dermatology and cosmetology [24], including the treatment of scars, stretch marks, alopecia [5] and skin rejuvenation [35]. According to a systematic review, PRP had some positive effect in the aesthetic treatment and correction of scars as well as androgenic alopecia (88% based on 21 studies) [7].

Most of the conducted studies have shown some positive local effects of PRP. However, there are studies, which demonstrated no positive clinical effect, especially in the later stages of the healing process [5, 22]. The long-term effects in the studies have not been achieved, suggesting that the effect of PRP is limited [24]. Also, the studies failed to include the patient-related factors, such as demographic variables (age, sex), the presence or absence of comorbidities, and any use of medication. All of these factors must be taken into account as they can cause changes in the body and therefore affect the quality of PRP received from patients. For example, there are differences in the level of cytokines and growth factors between older and younger patients, and higher levels of growth factors have been observed in women and those younger than 25 years [33]. In addition, the studies did not examine the systemic impact of PRP.

THE INFLUENCE OF PRP TRAINING METHODS ON ITS EFFICIENCY

Currently, various PRP preparation options are outlined. These techniques differ depending on the centrifugation time and the g force or revolutions per minute used, the number of platelets obtained, the type and number of growth factors and chemokines released, the number of leukocytes [13]. Consequently, selecting the best methods, kits, and procedures for PRP, or at least those, which are more or less adequate to treat different types of tissue damage, is very complex [18].

When sampling autologous PRP as an anticoagulant in various studies, heparin, EDTA, acid citrate dextrose or sodium citrate (the latter two are currently used more often) have been used [10].

The biological properties of PRP and its effectiveness in clinical trials are primarily affected by the PRP preparation methods. In clinical practice, two different methods are used:

1 – Standard blood cell separators: the separation technology is applied with hard and soft centrifugation steps in the bowl of a centrifuge with a continuous flow (used intraoperatively).

2 – Methods and Devices of gravity centrifugation (more commonly used technique). Centrifugation with high centrifugal force (g) is used to isolate the surface layer from a portion of blood containing platelets and leukocytes. The differences in g-strength, time, and centrifugation temperature result in the significant differences in yield, concentration, purity, viability, and ability to activate isolated platelets [10, 11].

To date, the technique of double low-speed centrifugation is considered to be a more effective technique [25].

The first stage of centrifugation is erythrocytes' separation. After the first centrifugation, the following three layers can be distinguished: the upper layer of serum with a small number of platelets, the middle layer of plasma rich in platelets and leukocytes, and the lower layer, mainly consisting of erythrocytes [1].

It is crucial to separate red blood cells as the membranes of erythrocytes in the PRP samples can be damaged, thereby releasing plasma-free hemoglobin (PFH). The PFH and its breakdown products (hemoglobin and iron) together have a harmful and cytotoxic effect on tissues, causing oxidative stress, loss of nitric oxide, activation of the inflammatory pathway and immune suppression. These effects eventually result in the micro-vasculatory dysfunction, local vasoconstriction with damage to blood vessels and tissues [10, 32].

After the first centrifugation, the upper and middle layers are transferred to a new empty sterile tube for the second centrifugation. Once done, the upper layer of the resulting plasma containing a small number of platelets is removed, and the lower layer enriched with platelets (PRP) is used in clinical practice [8].

The conducted systematic review together with a number of other studies showed that as a result of double centrifugation, the number of platelets and growth factors increases significantly (on average by 2-3 times) [1, 2, 15]. The number of platelets in the PRP after double centrifugation is about 3-5 times higher than in the blood [17, 20].

As for the optimal concentration of platelets for cell proliferation, the results of the studies vary greatly. Some researchers have proposed that higher doses of platelets in PRP generate an increased concentration of the growth factors, and accordingly accelerate tissue regeneration [13]. R. E. Marx was the first to demonstrate improved bone and soft tissue healing with a minimum platelet count of 1×10^6/μl in the PRP [26]. I. Giusti showed that a concentration of 1.5×10^6/μl is optimal for tissue repair, however, higher platelet concentrations reduced their angiogenic potential, thereby reducing the efficiency of PRP [16]. To date, there is no clear evidence that lower or higher platelet concentrations can reduce or enhance the positive effect of PRP administration [1]. The correlation between platelet dose and the concentration of released bioactive platelet growth factors cannot be controlled because there are marked differences in the baseline platelet count between individual patients [12] and differences between PRP preparation methods [10, 31].

One of the unresolved issues is platelet activation. PRP is activated by using calcium chloride (CaCl2), fibrin and/or thrombin, light activation [33]. Some researchers believe that PRP needs to be frozen as the freeze-thaw cycle activates PRP. During high-speed centrifugation (1,000–10,000 g for a long time) platelets are damaged, and thereby growth factors are released [34]. Few authors believe that PRP can be used without an activating agent because platelets are spontaneously activated when exposed to cutaneous collagen and thrombin after injection [3, 33].

In addition, age, sex, the presence or absence of comorbidities, and medication may affect the quality of autologous PRP. Higher levels of growth factors have been observed in women and those younger than 25 years of age [33]. In addition, different types of cells and tissues have different needs for growth factors or cytokines for their proliferation. This emphasizes the need to consider the tissue-specific requirements for the components of PRP products.

The heterogeneity of PRP preparations, both now and in the past, makes it difficult to interpret the existing literature and limits the ability to make definitive recommendations on the clinical efficacy of PRP [10, 23, 27].

CONCLUSION

These days, PRP is widely used in various fields of medicine, but there is no single standard algorithm for its preparation as well as no clear criteria for setting indications for this procedure. The PRP preparation methods vary in different studies, therefore adding some variability to the results obtained, by which it is difficult to judge the efficiency of the PRP's use. The following questions remain unresolved: an effective method for PRP preparation, the optimal concentration of platelets and growth factors, insufficiently studied molecular mechanisms of PRP to promote tissue regeneration as well as systemic reactions to the administered PRP. Further high-quality studies are required to solve all the questions raised.

Authors' contribution:

A. R. Koshkinbayeva, O. A. Ponamareva, B. K. Omarkulov, Ya. A. Yutskovskaya – concept and design of the study.

A. R. Koshkinbayeva, A. V. Ogizbayeva – collection and processing of materials.

A. R. Koshkinbayeva, A. V. Ogizbayeva – writing of the text.

B. K. Omarkulov, O. A. Ponamareva, Ya. A. Yutskovskaya – editing.

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А. Р. Кошкинбаева[1], А. В. Огизбаева[2], О. А. Понамарева[3], Б. К. Омаркулов[1], Я. А. Юцковская[4]*

ЭФФЕКТИВНОСТЬ И БЕЗОПАСНОСТЬ АУТОЛОГИЧНОЙ ПЛАЗМЫ, ОБОГАЩЕННОЙ ТРОМБОЦИТАМИ

[1]Кафедра семейной медицины НАО «Карагандинский Медицинский университет» (100008, Республика Казахстан, г. Караганда, ул. Гоголя, 40; e-mail: shar_surgeon89@mai.ru)
[2]Кафедра скорой медицинской помощи, анестезиологии и реаниматологии НАО «Карагандинский Медицинский университет» (100008, Республика Казахстан, г. Караганда, ул. Гоголя, 40; e-mail: eleusizova.a@kgmu.kz)
[3]Кафедра биомедицины НАО «Карагандинский Медицинский университет» (100008, Республика Казахстан, г. Караганда, ул. Гоголя, 40; e-mail: Ponamareva@qmu.kz)
[4]ООО «Клиника профессора Юцковской» (129626, Российская Федерация, г. Москва, вн. тер. г. муниципальный округ Алексеевский, проспект Мира, д.102, стр. 26, помещ. 8 Н/2; e-mail: ryazanova_lora@mail.ru)

[*]**Алина Робертовна Кошкинбаева** – PhD докторант, кафедра семейной медицины НАО «Карагандинский медицинский университет»; 100008, Республика Казахстан, г. Караганда, ул. Гоголя, 40; e-mail: shar_surgeon89@mai.ru

Целью данного литературного обзора является предоставление актуальных результатов исследований эффективности и обоснованности применения аутологичной плазмы, обогащенной тромбоцитами в различных сферах медицины, а также освещение нерешенных вопросов и проблем в данной сфере. По результатам поиска в базах данных *Scopus* и *Pubmed* по ключевым словам «platelet rich plasma», «safety», «effects» найдено 590 публикаций за последние 5 лет, большая часть из которых выполнена в сфере ортопедии, гинекологии и дерматологии.

Аутологичная плазма, обогащенная тромбоцитами (platelet-rich plasma) на сегодняшний день широко применяется в различных сферах медицины. Аутологичная плазма, обогащенная тромбоцитами, получила такое широкое применение, так она высвобождает многочисленные хемокины, цитокины и факторы роста, которые стимулируют ангиогенез, ремоделирование внеклеточного матрикса, а также дифференцировку и пролиферацию клеток. Множество проведенных исследований показывает положительные местные эффекты применения аутологичной плазмы, обогащенной тромбоцитами: более ранние васкуляризация, эпителизация, ремоделирование и регенерация тканей. Однако в настоящее время исследования по клинической эффективности аутологичной плазмы, обогащенной тромбоцитами, не являются окончательными и однородными. Гетерогенность препаратов аутологичной плазмы, обогащенной тромбоцитами (различные методики приготовления, разная концентрация тромбоцитов и факторов роста, влияние возраста, пола, сопутствующей патологии пациента), затрудняет интерпретацию существующей литературы и ограничивает способность давать окончательные рекомендации по клинической эффективности аутологичной плазмы, обогащенной тромбоцитами. Сегодня нет единого стандартного алгоритма подготовки аутологичной плазмы, обогащенной тромбоцитами, а также четких критериев для установки показаний к проведению данной процедуры. Остаются нерешенными вопросы: эффективного метода подготовки аутологичной плазмы, обогащенной тромбоцитами, оптимальной концентрации тромбоцитов и факторов роста, недостаточно изучены все молекулярные механизмы действия аутологичной плазмы, обогащенной тромбоцитами, способствующие регенерации тканей, а также системные реакции на введение аутологичной плазмы, обогащенной тромбоцитами. Необходимо дальнейшее проведение наиболее качественных исследований для решение всех поставленных вопросов.

Ключевые слова: обогащенная тромбоцитами плазма; эффективность; безопасность; факторы роста; регенерация

А. Р. Кошкинбаева[1*]*, А. В. Огизбаева*[2]*, О. А. Понамарева*[3]*, Б. К. Омаркулов*[1]*, Я. А. Юцковская*[4]

ТРОМБОЦИТТЕРГЕ БАЙ АУТОЛОГИЯЛЫҚ ПЛАЗМАНЫҢ ТИІМДІЛІГІ МЕН ҚАУІПСІЗДІГІ

[1]«Қарағанды медицина университеті» КЕАҚ Отбасылық медицина кафедрасы (100008, Қазақстан Республикасы, Қарағанды қ., Гоголь к-сі, 40; e-mail: shar_surgeon89@mai.ru)
[2]«Қарағанды медицина университеті» КЕАҚ Жедел медициналық көмек, анестезиология және реаниматология кафедрасы (100008, Қазақстан Республикасы, Қарағанды қ., Гоголь к-сі, 40; e-mail: eleusizova.a@kgmu.kz)
[3]«Қарағанды медицина университеті» КЕАҚ Биомедицина кафедрасы (100008, Қазақстан Республикасы, Қарағанды қ., Гоголь к-сі, 40; e-mail: Ponamareva@qmu.kz)
[4]«Профессор Юцковская клиникасы» ЖШС (129626, Ресей Федерациясы, Мәскеу, ішкі аумақтық муниципалды ауданы Алексеевский, Мира даңғылы, 102, 26 корпус, 8Н/2 бөлме; e-mail: ryazanova_lora@mail.ru)

***Алина Робертовна Кошкинбаева** – PhD докторанты, «Қарағанды медициналық университеті» КЕАҚ, Отбасылық медицина кафедрасы, 100008, Қазақстан Республикасы, Қарағанды қ., көш. Гоголь, 40; e-mail: shar_surgeon89@mai.ru

Бұл әдеби шолудың мақсаты медицинаның әртүрлі салаларында тромбоциттермен байытылған аутологиялық плазманы қолданудың тиімділігі мен негізділігін зерттеудің өзекті нәтижелерін ұсыну, сондай-ақ осы саладағы шешілмеген мәселелер мен мәселелерді қамту болып табылады. *Scopus* және *PubMed* дерекқорларында іздеу нәтижелері бойынша «platelet rich plasma», «safety», «effects» кілт сөздері бойынша соңғы 5 жылда 590 басылым табылды, олардың көпшілігі ортопедия, Гинекология және дерматология саласында орындалды.

Тромбоциттермен байытылған аутологиялық плазма бүгінгі күні медицинаның әртүрлі салаларында кеңінен қолданылады. Тромбоциттермен байытылған аутологиялық плазма осындай кең қолданысқа ие болды, сондықтан ол ангиогенезді, жасушадан тыс матрицаны қайта құруды және жасушалардың дифференциациясы мен көбеюін ынталандыратын көптеген химокиндерді, цитокиндерді және өсу факторларын шығарады. Жүргізілген көптеген зерттеулер тромбоциттермен байытылған аутологиялық плазманы қолданудың оң жергілікті әсерін көрсетеді: ертерек васкуляризация, эпителизация, тіндердің қайта құрылуы және регенерациясы. Алайда, қазіргі уақытта тромбоциттермен байытылған аутологиялық плазманың клиникалық тиімділігі туралы зерттеулер нақты және біртекті емес. Тромбоциттермен байытылған аутологиялық плазма препараттарының гетерогенділігі (дайындаудың әртүрлі әдістері, тромбоциттер мен өсу факторларының әртүрлі концентрациясы, жасының, жынысының әсері, науқастың ілеспе патологиясы) бар әдебиеттерді түсіндіруді қиындатады және тромбоциттермен байытылған аутологиялық плазманың клиникалық тиімділігі бойынша түпкілікті ұсыныстар беру мүмкіндігін шектейді. Бүгінгі таңда тромбоциттермен байытылған аутологиялық плазманы дайындаудың бірыңғай стандартты алгоритмі, сондай-ақ осы процедураның көрсеткіштерін орнатудың нақты критерийлері жоқ. Тромбоциттермен байытылған аутологиялық плазманы дайындаудың тиімді әдісі, тромбоциттердің оңтайлы концентрациясы және өсу факторлары, тіндердің регенерациясына ықпал ететін тромбоциттермен байытылған аутологиялық плазманың барлық молекулалық әсер ету механизмдері, сондай-ақ тромбоциттермен байытылған аутологиялық плазманы енгізуге жүйелік реакциялар жеткілікті зерттелмеген. Барлық қойылған мәселелерді шешу үшін неғұрлым сапалы зерттеулер жүргізу қажет.

Кілт сөздер: тромбоциттерге байытылған плазма; тиімділік; қауіпсіздік; өсу факторлары; регенерация