Platelet-rich plasma (PRP) is plasma with high concentration of platelets compared with whole blood. The therapeutic effect of platelet-rich plasma is based on the effect of growth factors contained in α-granules of platelets. Transforming growth factor β1 (TGF-β1) is a growth factor of TGF-β superfamily which an amount is considerable in platelets and have the important role in musculoskeletal system regeneration.

MATERIALS AND METHODS. In this study using the ELISA, we determined the content of TGF-β1 in platelet-rich plasma in 14 patients with various musculoskeletal disorders, aged from 21 to 79.

RESULTS. The level of TGF-β1 in platelet-rich plasma was found to be 194.57 ± 25.76 ng/ml, which was 30 times higher than its control content (platelet-poor plasma), where its content was 6.52 ± 3.26 ng/ml. No statistically significant difference was observed between TGF-β1 levels in platelet-rich plasma in the patients of different age and gender.

CONCLUSIONS. It has been established that platelet-rich plasma can serve as a source of TGF-β1 for therapeutic purposes. TGF-β1 content in platelet-rich plasma has been shown to be independent of gender and age and, therefore, a wide range of patients may be treated with it.

KEY WORDS: platelet-rich plasma; TGF-β1; diseases of the musculoskeletal system
ORIGINAL RESEARCH

MATERIALS AND METHODS

Autologous biotechnological products of the blood of 14 patients with various diseases and injuries of the musculoskeletal system became the material for the study. Patients had exclusively local pathology of the musculoskeletal system, without systemic concomitant diseases, so this sample can be considered homogeneous. The distribution of patients by types of pathology is given in Table 1. There were 7 women and 7 men aged 21 to 79 years among them, at the average age of 49 ± 22 years. The study involved women aged 21 to 79 years (average age 52 ± 25 years) and men aged 25–77 years (average age 48 ± 20 years). The patients provided an informed consent to the study.

To prepare PRP, we performed the sampling of 50 ml venous blood in vacuum tubes with anticoagulant citrate dextrose. Blood sampling was performed fasting. A week before blood sampling, non-steroidal anti-inflammatory drugs were discontinued, patients were assigned a special diet (exclusion of fatty, fried and spicy foods, fluid intake up to 3 liters per day, exclusion of coffee) and regimen (refusal of smoking and alcohol).

The blood was centrifuged at 250 xg for 10 min to separate plasma and blood cells using a centrifuge CM-3 (MICROmed, China). Thereafter, the plasma was transferred to new tubes and centrifuged at 2300 xg for 5 min. The pellet containing platelets was resuspended in 3 ml platelet-poor plasma (PPP) [4]. Platelet-poor plasma (PPP) obtained from the second plasma centrifugation was used as a control. 500 μl samples of PRP and PPP were frozen and stored at -20 °C.

For enzyme-linked immunosorbent assay (ELISA), the samples of PRP and PPP were thawed at room temperature and were centrifuged at 250 xg for 10 min. The supernatant was used to further study using the TGF-β1 ELISA kit (DRG International, Inc., USA) according to the manufacturer’s instructions. Optical density measurements were performed on a microplate reader Synergy HT SIAFRTD (Bio-Tek Instruments, USA) at a wavelength of 450 nm. According to the optical density of standards with known concentrations of TGF-β1, a calibration curve was designed, according to which the content of TGF-β1 in ng/ml was calculated.

The variables of experimental data were determined by descriptive statistics and are presented as mean ± standard deviation. The Student’s t-test was used as the criterion for the significance of differences between the groups. The differences were considered as statistically significant at p < 0.05. Statistical analysis of the data was performed using Excel statistics software (Microsoft, USA).

RESULTS AND DISCUSSION

The content of TGF-β1 was determined by ELISA in 23 samples of PRP obtained from 14 patients with various pathologies of the musculoskeletal system (Table 1). The blood of each patient as a donor was used to prepare from 1 to 3 PRP products without taking into account the pathology of the musculoskeletal system, age and gender.

It was determined that the average level of TGF-β1 in PRP samples was 194.57 ± 25.76 ng/ml compared to the control PPP group, where its content was 6.52 ± 3.26 ng/ml (Fig. 1). Accordingly, the level of TGF-β1 in PRP was 30 times higher than in control PPP.

TGF-β1 plays a key role at all stages of chondrogenesis [24] and is therefore considered as a potential therapeutic agent for osteoarthritis.

Table 1. Individual parameters of TGF-β1 content in platelet-rich (PRP) and platelet-poor plasma (PPP) in patients with different pathologies of musculoskeletal system.
Whereas, J. Evanson et al. found that people up to 25 years old had higher level of TGF-β1 in PRP does not depend on the age of the studied patients [25, 30]. The level of TGF-β1 was investigated in patients’ PRP, depending on their age without taking into account gender, since no significant difference was found between TGF-β1 content in platelet-enriched plasma (PRP) and platelet-poor plasma (PPP); p > 0.008. No statistically significant difference was found between TGF-β1 levels in platelet-rich plasma between different genders (p = 0.4). Therefore, TGF-β1 can be used to treat a variety of disorders of the musculoskeletal system while PRP serves as a source for therapeutic purposes.

Data on the dependence of TGF-β1 content in PRP on the age are quite contradictory. Some studies showed that the content of TGF-β1 in PRP does not depend on the age of the studied patients [25, 30]. Whereas, J. Evanson et al. found that people up to 25 years old had higher cytokine levels in PRP than those of older age groups [31].

CONCLUSION

1. In the study of TGF-β1 content in platelet-rich plasma, it was shown that its level was 30 times higher than in the control platelet-poor plasma samples. No statistically significant difference was found between TGF-β1 levels in platelet-rich plasma between different genders. No dependence of TGF-β1 content in platelet-rich plasma on patient age was found.

2. In vitro study determined that platelet-rich plasma can serve as a source of TGF-β1 for the treatment of various diseases of the musculoskeletal system. In this study, it was shown that the content of TGF-β1 in platelet-rich plasma was independent of the gender and age of the examined patients, and therefore may be recommended for use in a wide range of patients.

REFERENCES


