Progress on Platelet Immunology in Iranian Blood Transfusion Organization (IBTO)

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Many Researches and thesis related to platelet functions, quality control of platelet products, Platelet Receptors, GP shedding have been performed by several people in Iranian Blood Transfusion Organization (IBTO) but Platelet Immunology here is started since 1999.......
Our first study was about cytokine production during storage of platelet products prepared by platelet rich plasma (PRP) in IBTO, started since 1998 and the first report was published in Tehran university Medical Journal, 1383= 2004, in brief prestorage leukodepletion decreased IL-8, IL-6 and TNF-a level but their level was increased during storage in unfiltered, nonirradiated, and gammairradiated random-donor platelet concentrates.
Effect of Gamma Irradiation on Lymphocyte Proliferation and IL-8 Production by Lymphocytes Isolated from Platelet Concentrates

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Background. Gamma-irradiation of platelet concentrates may inactive contaminated lymphocytes and subsequently inhibit the synthesis of cytokine in platelet during storage. The aim of this study was to determine the effect of γ-irradiation on the production of IL-8 and lymphocyte proliferation isolated from random donor platelet concentrates during 3-day storage.

Methods. In this study, we evaluated the effect of γ-irradiation on both lymphocyte proliferative response and IL-8 production for 29 random donor platelet concentrates (RD-PCS). All PCs were prepared from single RD by platelet-rich plasma (PRP) method were divided in two groups: 1) non-irradiated RD-PCS (n = 13) and 2) irradiated RD-PCS (n = 16). The RD-PCS were treated by γ-irradiation (30 Gy) on days 0 and 3.

Results. IL-8 increased in both groups after 3 days. It showed increase in concentration of IL-8 in irradiated and non-irradiated PCs during storage. Results of lymphocyte transformation test (LTT) indicated that the proliferation activity of lymphocyte was inhibited by γ radiation.

Conclusions. These data indicate that γ irradiation inhibits proliferation of lymphocyte but does not inhibit production and accumulation of IL-8 during the storage of PCs. © 2008 IMSS. Published by Elsevier Inc.

Keywords: Interleukin-8, ELISA, γ-Irradiation, Proliferation, Lymphocyte transformation test, Platelet concentrates.
Flowcytometric evaluation of antibodies against histocompatibility antigens and platelet-specific antigens in patients with hematological disorders following the transfusion of platelets concentrates

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Abstract

Background and Objectives
Blood transfusion may lead to the manifestation of anti-HLA and platelet-specific antibodies that may in turn bring about different problems like platelet refractoriness. It appears that the study of antibodies against HLA-Class 1 and platelet-specific antigens are useful for the selection and success of the appropriate treatment protocol. The aim of this study was to detect anti-HLA and anti-platelet-specific antibodies by flowcytometry in patients with hematologic disorders (including Acute Leukemia, Aplastic Anemia) and patients with ITP.

Materials and Methods
In this descriptive study, anti-HLA and platelet-specific antibodies were detected by flowcytometric technique, using 82 sera drawn from patients with different hematological disorders who showed a poor response to platelet transfusion and 20 from patients with ITP. The results of anti-HLA antibodies were then compared by Panel Reactive Antibodies (PRA).

Results
Our results showed 44 (53.7%) out of 82 (53.7%) patients had anti-HLA Class-I antibodies in their sera. The frequency of each antibody isotype was found to be as follows: IgM (51.2%), IgG (32.9%) and IgA (1.2%). 36 (43.9%) out of 82 patients had platelet specific antibodies and the frequency of each antibody isotype was found to be as follows: IgM (40.2%), IgG (30.5%) and IgA (12.2%). 27 (31.7%) out of 82 patients had both antibodies. No difference was found between the two groups in platelet specific antibodies. Despite significant correlation between flowcytometry and PRA methods, PRA can only detect antibodies which react with complement.

Conclusions
With increase in the number of platelet transfusion, immunization to HLA antigens occurs; moreover, immunization against platelet specific antigens may also occur during autoimmunity. The presence of these antibodies may be one of the reasons of poor response to platelet transfusion and platelet refractoriness in patients under study. Conducting similar studies with higher number of samples, platelet cross-match, and the use of HLA-matched platelets for these patients are recommended.

Key words: HLA-antibodies, Hematological disorders, Flowcytometry, Platelet refractoriness, PRA, Platelet specific antibodies

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Platelet activation and IL-8 production in platelet concentrates prepared by buffy coat and PRP method

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Abstract
Background and Objectives
The process of platelet concentrate production by plasma rich (PRP) method could activate the platelet and granules secretion of beta thromboglobulin, LDH and CD62P. Platelets activated during the preparation process do not have sufficient efficiency for hemostasis in vivo. It seems that platelet preparation by buffy coat method has an ability less than PRP to activate the platelet. Measuring platelet activation indices, such as CD62P expression and beta thromboglobulin, is a useful means to evaluate the percentage of activated platelet concentrates and compare the two methods of buffy coat and PRP.

Materials and Methods
In this experimental study, 15 concentrates were prepared via PRP method and 15 via BC method; 15 intact blood units were also considered as control group. The percentages of CD62P expression, soluble CD62P concentrates, IL-8 level, and CD14 positive cells were evaluated. Special monoclonal antibodies that conjugated with fluorescence dye in flowcytometric method were used for CD62P and CD14. ELISA method was used for evaluation of soluble CD62P and IL-8.

Results
The average platelet count in both methods showed no significant difference, but WBC contamination rate in PRP-PCs was more than BC-PCs. In PRP-PCs, we found a little decrease in CD62P expression and increase in soluble form and IL-8 level during reservation time. The level of CD14 showed no significant difference in these components. In BC method during the three day reservation, expression of CD62P, its soluble form, and IL-8 concentrates increased and the level of monocyte surface CD14 showed slight decrease ranging from 0.4 to 0.1.

Conclusions
It is concluded that there is a close relationship between IL-8 and WBC count in platelet concentrates. In PRP method in contrary to BC method, high speed centrifuge causes adhesion, aggregation and platelet activation.

Key words: Platelet, Platelet rich plasma, CD62P, IL-8, CD14.
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Platelet antigens frequency in blood donors: comparison of molecular detection with ELISA method (for HPA-1a)

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Abstract

Background and Objectives
Serologic methods used in HPA-typing are limited due to restricted access to specific antisera and decreased platelet count in thrombocytopenic patients. Therefore, several DNA-based HPA-genotyping techniques were used to determine the genotype of HPAs. Since nothing is known about the HPA gene frequency in Iran, this study was performed to determine its frequency in some Iranian blood donors.

Materials and Methods
DNA was extracted from a 3-ml whole blood sample prepared from donations of 100 Iranian blood donors collected in EDTA-coated blood tubes. Human platelet (PLT) alloantigens (HPA)-1/2/3/4/5 and HPA-15 typing were performed by the Polymerase Chain Reaction – Sequence Specific Primer technique (PCR-SSP), and HPA-1a phenotyping was performed by ELISA method for 40% of samples.

Results
The frequencies of HPA genes were: HPA-1a 98%, HPA-1b 2%, HPA-2a 54%, HPA-2b 46%, HPA-3a 48%, HPA-3b 52%, HPA-4a 100%, HPA-5a 99%, HPA-5b 1%, HPA-15a 47%, and HPA-15b 53%. HPA-4b was not found. The frequencies of HPA phenotypes were determined to be: HPA1a1a 96%, HPA1a1b 4%, HPA2a2a 8%, HPA2a2b 92%, HPA3a3a 19%, HPA3a3b 59%, HPA3b3b 22%, HPA4a4a 100%, HPA5a5a 98%, HPA5b5b 2%, HPA15a15a 14%, HPA15a15b 67%, and HPA15b15b 19%. 40 HPA-1a phenotyping by ELISA showed 26 positive (OD > 0.5), 4 negative (OD < 0.3), and 10 indeterminate samples (0.3 < OD < 0.5).

Conclusions
No HPA-1b/b homozygous genotype similar to other Asian studies was found. Since HPA-1,-2,-5 frequencies in the population under study differ from the European Caucasian race, it seems that antibody production in our population might be different from other Caucasians. According to HPA frequencies, it seems that HPA-2b, HPA-5b and HPA-15b may induce posttransfusion purpura and platelet refractoriness which need further investigation.

Key words: Platelet antigens, Gene frequency, PCR, Iran

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Platelet Transfusion Outcome and Flow Cytometric Monocyte Phagocytic Assay (FMPA)

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Abstract
Background: This study was designed to evaluate platelet transfusion outcome via flow cytometric monocyte phagocytic assay (FMPA).
Method: Fifteen patients with a history of multiple platelet transfusions and fifteen controls were enrolled in this study. CMFDA-labeled platelets were incubated with patients’ sera and were finally incubated with monocytes in a tube and analyzed by flow cytometry. Monocytes that phagocytosed platelets were detected as a CMFDA-positive platelet population via monocyte gate. The FMPA results were compared with CCI results for the patients.
Result: The FMPA result correlated with 1-hour \( r = -0.885, P = 0.001 \) and 24-hour \( r = -0.84, P = 0.001 \) CCI. There is a significant difference in means of FMPA results between the patients with immune platelet refractoriness (68.46 ± 10.4%), non-refractory group (37.73 ± 15.21%) and the control group (18.27 ± 2.86%).
Conclusion: Our data showed that FMPA has good results in evaluation of platelet transfusion outcome and may be useful as an indicator of platelet transfusion response.

Keywords: CMFDA, flow cytometry, FMPA, platelet transfusion,
Two projects related to HPAs molecular typing in HCV infected patients and women with previous abortions were recently completed in our center.
Thanks for your attention

Thanks My Dear colleagues and The students to help to perform theses researches