Evaluation of a freezing protocol to set up a provincial bone marrow transplant unit using lymphocyte proliferation test

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Abstract

Background: Bone marrow transplant (BMT) is one of standard treatment modalities in advanced hemato-oncology. To set up a BMT unit, one of the important steps is to evaluate the stem cell cryopreservation process before using in clinical program. **Methods**: Twenty one bags of buffy coat were collected in routine blood donation using quadruple bag. Like stem cell product, buffy coat was processed according to the freezing protocol prepared. The products were kept in liquid nitrogen for 2 weeks. There were paired separate samples from the same donor kept in 4^0C-refrigerator for the same period of time using for control. As a surrogate for viability, lymphocytes were extracted. Viability test was carried out with lymphocyte proliferation test together with trypan blue staining. Measuring optical density of each lymphocyte containing well after stimulation, lymphocyte proliferation value (LPV) was obtained, represented for viable lymphocyte. LPV and percentage of viable cell from trypan blue staining were compared in each sample between the beginning and 2 weeks after freezing, frozen and non-frozen. **Results**: Comparing before and after cryopreservation, LPV was 2.064 ± 0.379 (mean$\pm$SD) and 1.913 ± 0.546, ($p=0.314$) and percentage of viable cell with trypan blue staining was 97.46 ± 5.20 and 86.37 ± 7.46, ($p<0.05$), respectively. At 2 weeks after collection, comparing between frozen product and control (non-frozen), LPV was 1.913 ± 0.546 and 0.486 ± 0.453, ($p<0.05$) and trypan blue staining result was 86.37 ± 7.46 and 78.30 ± 10.94, ($p<0.05$), respectively. **Conclusions**: Analysis of LPV obviously shows the efficacy of freezing protocol especially preservation of cellular proliferation function. Result of trypan blue staining also demonstrates the protocol efficacy above control with certain amount of cells died along the freezing process. Avoiding from authentic stem cell collection, model of freezing protocol evaluation using lymphocyte proliferation test from buffy coat seems feasible and probably suitable for newly-established provincial BMT unit.