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A TRYPAN BLUE ABSORBANCE ASSAY FOR DETERMINATION OF CELL VIABILITY

Cell viability may be judged by a variety of assays based on morphological changes, alterations in membrane permeability and/or physiological state (cytolysis or membrane leakage, mitochondrial activity, genomic and proteomic assays etc.). Dye exclusion methods are traditionally used to assess cell viability, with trypan blue being one of the most common. In trypan blue exclusion method, cell viability must be determined visually by counting the unstained/stained cells with a microscope.

In this work, we propose the determination of cell viability by trypan blue staining method with the following spectrophotometric detection.

Methods. Mononuclear cells isolated from heparinized peripheral blood of 10 healthy donors by ficoll-verografin gradient centrifugation (p = 1,077 g/cm^3, 30 min., 1500 rpm). The cell death was inducted by incubation of mononuclear cells with 0,05% solution of acetic acid (5 min., 37 °C). Cellular viability is determined by measuring the capacity of cells to exclude vital dye – trypan blue. Peripheral blood mononuclear cells (2×10^6/ml) were incubated with 0,2% solution of trypan blue (1:1) in buffered isotonic salt solution (pH 7,2 to 7,3). The cell viability was quantified in cell suspensions by a light microscope as well as in cell culture supernatants by spectrophotometer. The absorption was measured at 450 nm, the reabsorption – at 620 nm. The statistical analysis was carried out using Statistica 8.0. The statistical significance of the results was determined by Wilcoxon signed-rank test and Sperman`s rank coefficient correlation. The value are given as median (25÷75% procentile).

Results. The trypan blue exclusion test is based on the principle that live cells possess intact cell membranes that exclude certain dyes whereas dead cells do not. In view of this, viable cells had clear cytoplasm when observed under a microscope whereas a nonviable cell had a blue cytoplasm. The viability of nontreated cells was 80,0 (78,0÷86,0)% as well as the viability of acetic acid-treated cells was 47,0 (27,0÷50,0)% (p < 0,01). The intensity of cell culture supernatants absorbance at 450–570 nm was significantly lower in acetic acid-treated cells in comparison with untreated mononuclear cells (1,25 (0,75÷1,35) *vs.*1,43 (1,34÷1,45) a. u.). At that, the absorbance of 0,1% trypan blue clear solution was 1,51 (1,47÷1,54) a.u. The number of viable cells was positively correlated with intensity of cell supernatants absorbance (r_s = 0,88, p < 0,01). The mathematic model of cell viability calculation was based on the level of cell supernatants absorbance (X) and was as follows: [number of viable cells, %] = $203,57\times X_2 - 365,19\times X + 186,6$.

Conclusion. The spectrophotometric assay for determination of cell viability included mononuclear cells staining by 0,1% solution of trypan blue and measurement of cell supernatants absorbance at 450–620 nm.

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BREAST CANCER RISK FACTORS

Breast cancer is the most common malignancy among women and the second leading cause of mortality due to cancer worldwide. Rapidly increasing incidence of breast cancer is a new social challenge resulting from a spectrum of internal and external risk factors, which, although accepted as a feature of the early twenty-first century, are new for female sub-populations compared to the past. These include altered socio-economical conditions such as occupational exposure; rotating shift work; specific environmental factors (increased pollution and environmental toxicity, altered dietary habits, quality and composition of meals); as well as consequently shifted and/or adapted physiologic factors such as lower menarcheal age; late age of first full-term pregnancy, if any; shorter periods of breastfeeding; and later menopause.

The aim of this study was to evaluate the prevalence of internal and external risk factors in women diagnosed with breast cancer living in the territory of Minsk and the Minsk region. The subject of the study was a cohort of 100 women, between the ages of 21-55 years old, with a diagnosis of breast cancer. Age analyzing makes it possible to determine in which age group the highest incidence of breast cancer was observed. A survey method was chosen as the main method of study.

An electronic database was created through survey and analysis of medical records, and statistical analysis of the identified factors that make the greatest contribution to the genesis of breast cancer.

Looking at these results, we can conclude that the most widespread risk factors of breast cancer in the study group are: reduction of the lactation period, which was observed in 28 (73,68±7,14%) of 38 (45,78±5,46%) women who breastfed their children; the presence of abortions in anamnesis: induced abortions occurring in 35 (77,7±6,19%) of 45 (54,21±5,46%) women whose pregnancy ended in childbirth; and in 10 (22,2±6,19%) of 45 (54,21±5,46%) women whose pregnancy ended in miscarriage.

In conclusion, the creation of individual patient profiles and regulation of modifiable risk factors may be the most optimal predictive and preventive strategy.