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Non-invasive mass spectrometric viability assessment of *in vitro* fertilized embryos using the alpha-1 chain of human haptoglobin

Gergely Montskó, Krisztina Gödöny, Ákos Várnagy, József Bódis and Gábor L Kovács
University of Pécs, Hungary

Infertility nowadays is a growing health issue in the developed world meaning that every year more and more couples visit an assisted reproduction (ART) centre. However, the success rate of the process is stagnating at about 30%. An effort is made worldwide to find new additional indicators of embryo viability to implement the routinely used morphological evaluation. Spent embryo culture medium samples (n=201) were measured using liquid chromatography coupled mass spectrometry in a series of retrospective, blind experiments. No sample preparation was made, 10 µl of sample was directly injected into the instrument after the addition of internal standard solution. A protein marker was found which significantly ($p<0.001$) differed in quantity between the samples of embryos which did or did not implant. This protein was identified as the alpha-1 chain of human haptoglobin molecule. A significant correlation ($p<0.001$) was also found when comparing the clinical outcome (clinical pregnancy or no pregnancy) and the outcome predicted by the measurements. The haptoglobin fragment quantitation serves as an additional tool along the process of morphological evaluation. The blind, retrospective results provided a positive predictive value of more than 50%. The negative predictive value of the analysis was 100%, there were no embryos which were diagnosed as "viable" but resulted in clinical pregnancy. The results provided a contra selection tool, screening the embryos with good morphological aspects, but no implantation potential.

e: montsko.gergely@pte.hu

The immunopathology of regression in keratoacanthoma

Fathia A Bayoumi
Medcare Hospital, UAE

Background*:* Keratoacanthoma (KA) has a tendency for either progression or spontaneous regression. Regression is a phenomenon present in a variety of cutaneous lesions. It is likely that certain immunologic mechanisms explain the phenomenon of spontaneous regression occurring in KA. Causes and detailed mechanism of this regression are still not completely elucidated. Recent studies suggested that the tumor regression is dependent mainly on the stromal immune response.

Aim*:* As a first step in confirming or refuting this hypothesis, we did an immunohistochemical study of KA. Also, we correlated between the tumor size, rate of proliferation and stromal infiltration by cytotoxic T lymphocytes which release granzyme-B.

Methods & Results*:* This is a case series study done on 20 cases of KA that were examined and clinicopathological findings were reviewed. Immunohistochemical stains using PCNA, P53 and granzyme-B were done. PCNA showed positive staining in all cases (100%) with significant positive correlation with the tumor size (0.5, $p<0.02$). P53 was positive in 16 cases (80%) with highly significant positive correlation with the tumor size (0.63, $p<0.0028$). Granzyme-B was positive in the stromal lymphocytes and histiocytes only in 6 cases (30%) with highly significant negative correlation with the tumor size (-0.79, $p<0.0001$). Negative correlation between PCNA overall score and granzyme-B was evident (-0.37) and between P53 overall score and granzyme-B also (-0.38). The mean total score for granzyme-B was higher (1.04+0.23) in tumors less than 1 cm in size if compared with that in tumors more that 1cm in size (0.66+0.12).

Conclusion*:* The increased release and/or activity of granzyme-B as CTL-mediated response were a central effector mechanism in tumor regression in KA.

e: drfatehia@hotmail.com