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Proteomics analysis of cytotoxic factors of periapical exudate using 2-D electrophoresis

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I. Object

The aim of this study was to evaluate possible different protein-expression patterns, and to evaluate their relevance to the apical exudates from patients who had periapical lesion in their radiographs.

II. Materials & Methods

The present study included patients who had been referred for root canal treatment to the Department of Conservative Dentistry, Dankook Dental Hospital. Samples were taken from the root canal s of the teeth having necrotic pulp, radiographic evidence of periradicular diseases. Two-dimensional gel electrophoresis of collected exudates was performed as follows: 60㎍ of protein was applied onto IPG strips, containing immobilines(pH 3-10), thioruea, CHAPS, and urea. After isoelectric focusing, the strip was placed on top of a sodium dodecyl sulfate(SDS)-poly-acylamide gel(12 ㎝ × 14 ㎝, 12.5%) and proteins were separated, according to molecular weight, in a horizontal apparatus. Spots were detected either by silver staining or by colloidal Coomassie Brillant blue G-250, for tryptic digestion and protein identification. Spectra were processed and analyzed by using the Global Protein Server Workstation, which uses internal MASCOT software for searching the peptide mass fingerprints.

III. Results

Comparison of the 2D gel electrophoresis patterns obtained from 3 different teeth showed a similar pattern of protein distribution for all samples from all subjects. The number of spots observed on the 2D gel electrophoresis studies was about 50 spots. It was observed that a higher number of spots in the high molecular weight area.

IV. Conclusion

On the basis of analysis of cytotoxic factors using 2-D electrophoresis, a variety of proteins such as queuine tRNA-ribosyl transferase, alpha-1-antitrypsin, Carbonic anhydrase I were obtained. And the two-dimentional electrophoresis study was a useful tool to identify the cytotoxic factor of the periapical substracts.