# Human Exoproteome in Acute Apical Abscesses

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## Abstract

**Introduction:** An acute apical abscess is a severe response of the host to massive invasion of the periapical tissues by bacteria from infected root canals. Although many studies have investigated the microbiota involved in the process, information on the host factors released during abscess formation is scarce. The purpose of this study was to describe the human exoproteome in samples from acute apical abscesses. **Methods:** Fourteen pus samples were obtained by aspiration from patients with an acute apical abscess. Samples were subjected to protein digestion, and the tryptic peptides were analyzed using a mass spectrometer and ion trap instrument. The human proteins identified in this analysis were classified into different functional categories. **Results:** A total of 303 proteins were identified. Most of these proteins were involved in cellular and metabolic processes. Immune system proteins were also very frequent and included immunoglobulins, S100 proteins, complement proteins, and heat shock proteins. Polymorphonuclear neutrophil proteins were also commonly detected, including myeloperoxidases, defensins, elastases, and gelatinases. Iron-sequestering proteins including transferrin and lactoferrin/lactotransferrin were found in many samples. **Conclusions:** The human exoproteome included a wide variety of proteins related to cellular processes, metabolism, and immune response. Proteins involved in different mechanisms against infection, tissue damage, and protection against tissue damage were identified. Knowledge of the presence and function of these proteins using proteomics provides an insight into the complex host-pathogen relationship, the host antimicrobial strategies to fight infections, and the disease pathogenesis. *(J Endod 2017;■:1–7)*

## Key Words

Acute apical abscess, endodontic infection, exoproteome, immune defense

## Significance

In acute apical abscesses, the host cells released a large array of proteins, many of them related to the immune response against infection. Host defense proteins included neutrophil-related proteins, immunoglobulins, complement, and iron-sequestering proteins. Proteins associated with tissue damage and protection against tissue damage were also identified. Abscesses result from a complex host-pathogen interaction characterized by the release of proteins with a broad diversity of functions.

Endodontic infections are polymicrobial in nature and are the primary cause of apical periodontitis (1, 2). An acute apical abscess is a severe manifestation of apical periodontitis and one of the most common and severe dental infections (3). This type of abscess is characterized by an extraradicular infection resulting from the massive egression of bacteria and their products from the necrotic root canal to the apical periodontal tissues. The severity of tissue damage depends on the bacterial counts and virulence factors as well as the host response; actually, in abscesses, the latter may cause the most significant damage to the tissues in response to bacterial infection (4). In some cases, endodontic abscesses can lead to complicated, sometimes life-threatening conditions (5–7).

The microbiota associated with acute apical abscesses has been extensively studied by culture-dependent and culture-independent studies (8). Bacteria belonging to the genera *Porphyromonas*, *Fusobacterium*, *Treponema*, *Streptococcus*, *Parvimonas*, *Prevotella*, and *Dialister* have been found to prevail in samples from acute apical abscesses (9–12). Nonetheless, the host side of the process has not been studied in the same magnitude, and information about the host factors released in the course of an apical abscess is scarce.

Because acute abscesses represent a dramatic outcome of host-pathogen interaction, it is of great importance to identify the host factors released in the process. Metaproteomics has emerged as a large-scale evaluation of proteins that are differentially expressed in an environment and allows for improved understanding of the overall physiology of cells and tissues under certain conditions (13). In endodontics, metaproteomics has been used to identify the bacterial proteins associated with different manifestations of apical periodontitis, providing an insight into the physiological and pathogenic activity of the bacterial community (14–16). Only 2 studies have evaluated the host proteins expressed in response to endodontic infection (15, 16). Abscesses were examined in only 1 of these studies but were restricted to 2 cases (15). Knowledge of the host cell behavior in active infections, including abscesses, is essential to understand the disease pathogenesis. Therefore, the purpose of the present study was to evaluate the host exoproteome in pus samples taken from acute apical abscesses.

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0099-2399/$ - see front matter

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http://dx.doi.org/10.1016/j.joen.2017.04.019

## Materials and Methods

### Patient Selection and Sample Collection

The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all individuals. Samples were taken from 16 patients presenting to the dental clinic of the endodontic department for emergency treatment of teeth with acute apical abscesses over a period of 1 year. Samples from 2 patients were excluded because of the excessive presence of blood or analytical problems in protein quantification. Therefore, 14 patients (mean age, 42 years; range, 20–65 years) were included in the study. All 9 anterior and 5 posterior teeth had pulp necrosis as determined by pulp vitality tests. Patients complained of pain and presented with localized swelling. Two patients had additional signs/symptoms, including fever, lymphadenopathy, and malaise. Patient data are shown in Supplemental Table S1 (Supplemental Table S1 is available online at www.jendodon.com). Subjects with significant gingival recession and periodontal pockets deeper than 4 mm, systemic disease, and who had used antibiotics over the last 3 months were excluded from the study.

The overlying mucosa of each abscess was disinfected with 2% chlorhexidine, and samples were taken intraorally by aspiration of the purulent exudate using a 16-G sterile needle. Pus samples were immediately placed within cryotubes and frozen at −20°C until analysis.

### Protein Concentration Determination

Samples were thawed at room temperature and centrifuged at 9000*g* (4°C) for 15 minutes. After centrifugation, the supernatant was separated from the sediment and used in subsequent analyses. The protein concentration was determined using the Bradford method (17) and a standard curve with known concentrations of bovine serum albumin. Protein quantification was performed in triplicate using 96-well microplates. For this purpose, 1 $\mu$L of each sample (protein extract) diluted in 39 $\mu$L purified water was added to 160 $\mu$L 1.25× Bradford reagent. After 10 minutes at room temperature, absorbance was read at 595 nm using a microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, MA). A standard curve was obtained to determine the protein concentration of each sample.

### In-solution Trypsin Digestion

Samples were subjected to protein digestion using a filter-aided approach, which combines the advantages of in-gel and in-solution digestion for mass spectrometry as described elsewhere (18). An aliquot of 100 $\mu$g of each protein extract was solubilized in 400 $\mu$L 0.05 mol/L ammonium bicarbonate. Samples were reduced using 10 $\mu$L dithiothreitol and alkylated with 40 $\mu$L iodoacetamide for 1 hour at room temperature. Then, they were transferred to a Vivaspin 500 centrifugal filter unit (GE Healthcare, Uppsala, Sweden), solubilized with 400 $\mu$L 0.05 mol/L ammonium bicarbonate under vortexing, and centrifuged at 10,000*g* for 10 minutes. Protein samples were digested with trypsin (1:50, w/w) (Trypsin Gold, Mass Spectrometry Grade [V5280]; Promega, Madison, WI) overnight at 37°C. Digested samples were centrifuged and dried at 10,000*g* for 20 minutes.

### Mass Spectrometric Analysis

The tryptic peptides were solubilized in an aqueous solution of 0.1% formic acid and centrifuged at 20,000*g* for 20 minutes, and a 75-$\mu$L aliquot was transferred to a test tube. The mass spectra were acquired using 2 strategies. First, 20 $\mu$L of each sample was applied to a liquid chromatography–mass spectrometric system consisting of an ultra-performance liquid chromatography and a micrOTOF QII quadrupole time-of-flight (QTOF) mass spectrometer (Bruker Daltonics, Bremen, Germany). Samples were loaded onto a trap column and the ProteCol $C_{18}$ capillary column GHQ303 (300 $\mu$m × 150 mm) (Waters, Milford, MA), and elution was performed at a flow rate of 4.5 $\mu$L/min. The eluted peptides were injected into the micrOTOF-Q mass spectrometer using a microESI ionization needle (New Objective, Woburn, MA). The mobile phase buffers used for the gradient program were 0.1 % (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B). The gradient program consisted of 5% B for 5 minutes, linear ramping to 50% B over 35 minutes, linear ramping to 95% B over 10 minutes, holding at 95% B for 10 minutes, ramping back to 5% B over 5 minutes, and holding at 5% B for 5 minutes. Precursor ion scanning was performed in the positive mode in the mass range between 100 and 2000 m/z for the precursor ions (MS1) and between 70 and 2000 m/z for the product ions (MS2). Data were acquired for approximately 60 minutes. The QTOF was operated in the automatic mode (autoMSn) to acquire the MS1 and MS2 spectra. After the end of the analysis using the micrOTOF QII, 10 $\mu$L of each sample was applied to another liquid chromatography–mass spectrometric system composed of nanoACQUITY ultra-performance liquid chromatography (Waters, Milford, MA) coupled to the mass spectrometer Amazon Ion Trap (Bruker Daltonics). Samples were loaded onto a trap column and a $C_{18}$ BEH130 capillary column (1.7–100 $\mu$m × 100 mm), and elution was performed at a flow rate of 0.400 $\mu$L/min. Peptides were eluted automatically and injected online into the mass spectrometer through a nanoESI ionization needle. The same gradients used for peptide elution in the QTOF were used in the ion trap. Precursor ions were scanned in the positive ion mode in the mass range between 300 and 1500 m/z for MS1 spectra and between 70 and 3000 m/z for the MS2 spectra. Data were acquired for approximately 50 minutes. The ion trap was operated in the MSn mode to acquire MS2 spectra for the most intense ions in each full scan spectrum, and singly charged ions were excluded.

Data acquisition in the QTOF and ion trap was managed using Hystar software version 3.2 (Bruker Daltonics), and the spectra were analyzed using Data Analysis version 4.0 (Bruker Daltonics) and CompassXport version 3.0 (Bruker Daltonics) with the default settings for proteomics. The peak lists were generated in the mascot generic format (*.mgf) and the extensible markup language (*.mzXML) format using Data Analysis and CompassXport software, respectively.

### The Identification and Classification of Proteins

The mass data in the mzXML format were analyzed using the PEAKS software version 7.0 (Bioinformatics Solutions, Waterloo, ON, Canada), and the spectra were searched against a *Homo sapiens* database downloaded from UniProt in July 2016. The parameters used in the search were tryptic digestion with 1 missed cleavage, cysteine carbamidomethylation as a fixed modification, and methionine oxidation as a variable modification. For the ion trap, the error tolerance was 0.2 d for parent and fragment ions. For the QTOF, the error tolerance was 30 ppm for parent ions and 0.02 d for fragment ions. Proteins were identified when they presented at least 1 unique peptide with a false discovery rate of less than 1%. Each identified protein should have at least 1 peptide sequence not shared with any other protein (ie, unique peptides).

The identified human proteins were categorized according to their biological function into cellular processes and metabolism, circulatory system, extracellular matrix, immune system, and other/unknown, as previously described (15). Data were deposited and are publicly available at Peptide Atlas (http://www.peptideatlas.org/PASS/PASS00958).

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