Proteomic Analysis of Gingival Crevicular Fluid During Tooth Eruption

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Received: 05.12.2022 **Accepted:** 02.01.2023

ABSTRACT

Objective: Gingival crevicular fluid (GCF) is a biological fluid that has the unique capacity to reflect changes in periodontium to its protein composition, making it ideal for potential biomarkers. There is limited information about the mechanism of tooth eruption, for which GCF might provide valuable knowledge. This study aimed to provide a proteomic approach to investigate the composition of GCF obtained from two different supraosseous tooth eruption stages of permanent molars changes.

Methods: GCF samples were taken from a total of 26 healthy children, whose permanent molar just emerged from the gingiva (5-8 years old) and the occlusal equilibrium stage (9-13 years old). Proteins were extracted with Bio-Rad Rehydration Buffer followed by Zeba™ Spin Desalting Column. GCF samples were separated with two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by mass spectrometry-based protein identification.

Results: A new optimized protocol with enriched protein extraction from GCF samples was developed. Several proteins related to tooth eruption were detected. It was determined that keratin type II cytoskeletal 4 and keratin type I cytoskeletal 9 decreased and albumin increased in GCF protein content in erupting teeth compared to those in the occlusal equilibrium phase.

Conclusion: This methodology, which we have applied for 2D-PAGE of GCF, can also be a source for other studies. There is huge diagnostic potential in mass spectrometry technologies, this study can be carried forward by using other approaches.

Keywords: Tooth eruption, gingival crevicular fluid, proteomic analysis, two-dimensional gel electrophoresis, mass spectrometry.

1. INTRODUCTION

Tooth eruption is defined as all the movements that occur from the area where the tooth develops within the dentoalveolar structure until it reaches its functional position in the oral cavity (1). The process begins with the formation of primary teeth in the sixth week of intrauterine life and after the eruption and occlusal balancing of the third molars, it continues passively for a lifetime (1). Steedle and Proffit (2) divided eruptive movements into 6 phases, three pre-functional stages and three post-functional stages. They are classified as follicular growth, pre-emergent eruptive spurt, post-emergent eruptive spurt, juvenile occlusal equilibrium, circumpubertal occlusal eruptive spurt, and adult occlusal equilibrium (2). Most of our knowledge of the tooth eruption mechanism is based on animal studies, and although they provide very valuable information, they cannot directly reflect the process in humans (3).

The gingival crevicular fluid (GCF) is a biological fluid in the gingival sulcus surrounding the teeth, with a tooth on one side and epithelium on the other side, and is present as a transudate or inflammatory exudate originating from blood plasma (4). GCF has received major attention due to its unique capacity to reflect changes in the gingival area to its protein composition (4). It locally generates materials such as inflammatory mediators, host inflammatory cells, locally produced extracellular proteins, microbial plaque, and antibodies directed against dental plaque bacteria (5). Although various methods have been described for the GCF collection, such as capillary tube and gingival washing, the paper strip method is the most frequently employed one due to its easy and fast usage and is considered a non-invasive technique (6).

Although the development of mass spectrometry (MS) technology has led to extensive proteome documentation of

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DOI: 10.33808/clinexphealthsci.1214974

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body fluids such as plasma, whole saliva, parotid secretion, or minor gland secretion saliva in the dental field, the number of large-scale proteome analysis studies for GCF components is still very limited (4). One of the main reasons that biochemical or MS studies of GCF protein content are limited is because GCF is present in very small quantities (0.2 – 0.5 µL per site) in the healthy periodontium of adults (4). Other limitations are dynamic protein range and highly abundant protein content (4). Abundant proteins such as albumin or immunoglobins restrict the identification of low-level proteins (4).

With the progression of MS technologies, large-scale data on the protein content of GCF have been shown but most studies have focused on finding markers for periodontal diseases among adults (4,7,8). Few proteomic studies have been performed with GCF of pediatric subjects. To the best of our knowledge, proteomic analysis of GCF about tooth eruption has not been studied before. Tooth eruption is a complex and multifactorial process that is still not fully understood. Multiple tissue changes occur during this process, such as bone apposition and resorption, and the development of root and periodontium. One of the main hypotheses states that periodontal ligaments promote eruption by generating tension and compression of collagen fibers and fibroblasts (9), which makes us consider that some information about eruption may be concealed in the gingival sulcus pocket. We conducted two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of GCF obtained from apparently partially erupted permanent molars as compared with fully erupted permanent molars. This study aimed to investigate how the protein level in GCF changes during two different tooth eruption stages of permanent mandibular molars. And an additional aim is to contribute to the development of the 2D-PAGE protocol for GCF.

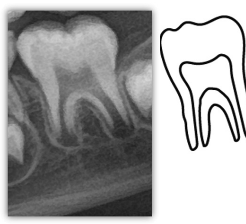
2. METHODS

2.1. Subjects

This study was approved by the Marmara University Faculty of Dentistry Clinical Research Ethics Committee (2019-271). Informed consent was obtained from each parent of pediatric subjects (26 children, 14 girls, 12 boys) who applied to Marmara University Faculty of Dentistry Department of Pediatric Dentistry. They were selected to participate in this study based on the following inclusion criteria; having general systemic and mental health, as well as healthy oral soft tissues and periodontal health (Silness-Löe Plaque Index (PI) score 0 or 1), not taking any antibiotics in the last 30 days, and absence of any orthodontic appliances, crowns or any tooth with pulpal pathology. Only right and left permanent mandibular first molars without any caries, sealants, fillings, or enamel hypoplasia were sampled (#36 and #46). Subjects abstained from brushing their teeth, chewing gum, eating, or drinking for at least 1.5 h before the visit. Subjects were examined in two groups according to the eruption stage of the tooth classified by Steedle and Proffit (2):

1 – Eruption Group (ER): Children aged between 5-8, whose permanent mandibular first molar tooth is just emergence the gingiva, clinically at least half of the molar tooth's occlusal surface is covered with gums and a radiologically wide-open-apex. It corresponds to the beginning of the post-emergent eruptive spurt phase (2) (Fig.1).

2 – Occlusal Equilibrium Group (OE): Children aged between 9-13 whose permanent mandibular first molar tooth fully erupted, clinically reach occlusion and contact with its antagonist tooth, and radiologically root length developed and narrowed apex. It corresponds to the beginning of the juvenile occlusal equilibrium phase (2) (Fig. 1).



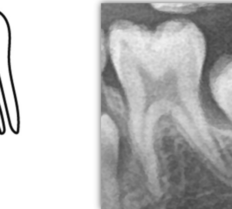
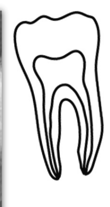



Figure 1. *Radiographic images and illustrations of subject groups: Eruption Group (ER) and Occlusal Equilibrium Group (OE).*

Before sample collection, an intra-oral examination was performed by a single investigator (SYA) under the reflector light. For periodontal evaluation, PI was evaluated by dryness of the teeth before the examination. After sample collection with paper strips, probing pocket depth (PPD) of molars was measured from mesiobuccal point of the sampling tooth with a Williams-type periodontal probe (0103.A0.01, Dentag, Maniago, Italy).

2.2. GCF Study Groups and Sample Collection

This study was carried out with eruption group subjects (n=3) and occlusal equilibrium group subjects (n=3), care was taken to ensure that factors such as age, gender, and tooth number were similar. GCF sampling site was first irrigated with 20 mL of isotonic saline (NaCl 0.9%, Polifarma, Tekirdag, Turkiye). GCF sample was collected with a sterile Periopaper strip (Oraflow Inc., New York, U.S.A.) by using the intrasulcular method after the site was protected from saliva contamination by cotton rolls (10). The Periopaper strip was gently inserted into the buccal sulcus until minimum resistance was sensed (1 mm) and left in place for 40 seconds (Fig. 2). Samples observed to be contaminated with blood or saliva were discarded. Clean Periopaper strips were placed in Eppendorf tubes which were stored at – 80 °C for further processing.

2.3. GCF Protein Extraction

All studies after sampling were carried out at Yeditepe University Proteomics and Mass Spectrometry Laboratory (YediPROT). Apical 2-3 mm of each collected Periopaper strip was cut with sterile scissors and placed in Bio-Rad Rehydration Buffer at 20 °C for 10 minutes. Zeba™ Spin Desalting Column (7K MWCO, Thermo Fisher, U.S.A.) was employed to remove any impurities from the protein sample that might affect further analysis following Zeba™ Spin instructions (Fig. 2).

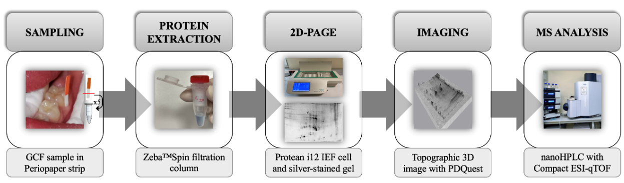


Figure 2. *Study Workflow*

2.4. Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE)

Approximately 130 µL of GCF protein sample was rehydrated onto ReadyStrip™ immobilized gradient strips (IPG Strips, 11cm pH 4-7, Bio-Rad, U.S.A.) under mineral oil at 20 °C for 16 h. Next, isoelectric focusing (IEF) was performed with Protean i12 IEF Cell (Bio-Rad, U.S.A.) (Fig. 2) following the protocol given in Table 1. Precast 8-16% polyacrylamide gels (Bio-Rad Criterion TGX Stain-Free Protein Gels, U.S.A.) were chosen for the second dimension with 1:10 Precision Plus Protein™ WesternC™ Standards (Bio-Rad, U.S.A.). GCF proteins were separated with SDS-PAGE and visualized with ProteoSilver™ Silver Stain Kit (Sigma-Aldrich Inc., U.S.A.).

Table 1. *Isoelectric focusing running protocol*

Step	Voltage [V]	Gradient	Current [µA]	Time [HH:MM]	Volt Hours
1	250	Rapid	50	0:20	HH:MM
2	8000	Gradual	50	1:00	HH:MM
3	8000	Rapid	50	26000	Volt Hr
4	750	Hold	50		

µA: Microampere, H:Hour, M:Minute

2.5. Gel Image Processing

Silver-stained gels were scanned using ChemiDoc XRS+ (Bio-Rad, U.S.A.) and analyzed by the PDQuest software (Ver.8.0.1.55 Bio-Rad, U.S.A.) (Fig. 2). Eruption group was called as ER (n=3) and occlusal equilibrium group as OE (n=3). Background subtraction and protein spot detection were processed automatically.

2.6. MS Analysis

The protein spots selected by the PDQuest were diced into smaller pieces to be destained completely by using 15 mM potassium ferricyanide and 50 mM sodium thiosulfate. Next, protein samples were reduced with 10 mM dithiothreitol and alkylated with 100 mM iodoacetamide under dark for 30 min. Following, protein samples were digested with trypsin (MS Grade – Gold Promega, Madison, WI, U.S.A.) at 37 °C for 16 h. The peptide samples were extracted and concentrated by using vacuum centrifugation. They were introduced into Thermo Dionex UltiMate™ 3000 RSLCnano using a pre-concentration setup with Acclaim PepMap RSLC C18, 2 µm, 100Å column (Mobile phase A: 100% H2O + 0.1% formic acid, Mobile phase B: 100% acetonitrile + 0.1% formic acid). This separation was followed by the analysis with NanoBooster Captivespray™ UHR Quadrupole Time-of-Flight (Bruker Compact, Germany). HyStar program for the control of the whole process, otofControl for MS, Compass Data Analysis for the generation of .mgf files and mascot-based SwissProt database search, and Biotools to list the proteins identified were employed to finalize the analysis.

3. RESULTS

3.1. GCF Study Groups

The mean age, gender, sampled tooth number, PI and PPD values of the two groups are given in Table 2. A total of 6 gels were run by repeating two groups in triplicate, and 5 Periopaper strip samples were used for each gel. The mean age was 6.84 years in the eruption groups, and 10.19 years in the occlusal equilibrium groups.

Table 2. *The mean age, gender, sampled tooth number, mean PI scores of two groups*

Eruption Group	n	Gender (F/M)	Mean Age (Week)	Tooth No (36/46)	PI Mean	PPD Mean
ER-1	5	2/3	356.0	2/3	0	0.9
ER-2	5	3/2	355.6	3/2	0	0.8
ER-3	5	3/2	358.4	3/2	0	0.7
Total	15	53% F 47% M	356.67 (age*:6.84)	53% #36 47% #46	0	0.8
Occlusal Equilibrium Group	**n**	**Gender (F/M)**	**Mean Age (Week)**	**Tooth No (36/46)**	**PI Mean**	**PPD Mean**
OE-1	5	2/3	528.4	3/2	0.6	0.8
OE-2	5	2/3	530.2	2/3	0.4	0.7
OE-3	5	3/2	535.8	2/3	0	0.6
Total	15	47% F 53% M	531.47 (age*:10.19)	47% #36 53% #46	0.33	0.7

*ER:Eruption Group, OE:Occlusal Equilibrium Group, F:Female, M:Male, PI: Silness-Löe Plaque Index, PPD: Probing Pocket Depth *1 year is calculated as 52.17 weeks.*

3.2. Optimization for Protein Extraction from GCF Samples

To develop an optimized protein extraction protocol, urea buffer and rehydration buffer (Bio-Rad ReadyPrep 2-D Rehydration Buffer, U.S.A.) were selected for the first step of this study. We evaluated the ability of extraction of these two methods by one-dimensional SDS-PAGE, and we observed

more diversified protein bands with rehydration buffer as a protein extraction solution (Fig. 3a).

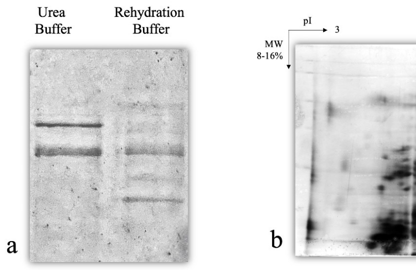


Figure 3. *a. One-dimensional SDS-PAGE gel image of two different extraction buffers; Urea buffer and Bio-Rad Rehydration Buffer b. Silver stained two-dimensional SDS-PAGE gel image of GCF sample, using wide range IPG strips (pH 3-10).*

3.3. Two-Dimensional Polyacrylamide Gel Electrophoresis

For the first dimension, the IEF procedure, a total of 4 hours and 40 minutes was followed. After the second dimension was performed, gels were stained with silver, and these 2D-PAGE gels were scanned, and their visual gel images are displayed in Fig. 4. These images demonstrate that the majority of the proteins detected have a low molecular weight below 50 kDa, and the majority of these proteins aggregated in the pH range of 5 to 6.5.

3.4. Gel Image Processing

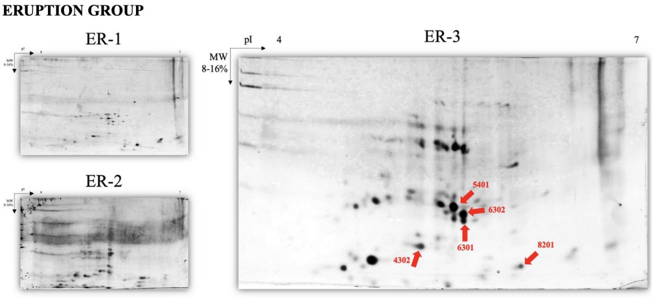
There were two groups with 3 replicates, Gel ER-3 was chosen as the master gel and all other gel-to-gel spot matchings were performed against this gel in PDQuest software. A greater number of spots were detected in the OE group (avg. 89) than ER group (avg. 67.6). It was found 86 protein spots were in the master gel, and 62% and 69% of all spots matched in group ER while group OE this rate resulted as 36%, 28% and 25% (Table 3).

Table 3. *Detected spots on each gel and their matched spot number, rate and correlation coefficient to master gel.*

Gel Name	Group	Spots	Matched	Match Rate-1**	Match Rate-2***	Correlation Coefficient
ER-1	ER	55	38	69%	43%	0.292
ER-2	ER	62	39	62%	44%	0.389
ER-3*	ER	86	86	100%	97%	1.000
OE-1	OE	95	27	28%	30%	0.314
OE-2	OE	101	26	25%	29%	N/A
OE-3	OE	71	26	36%	29%	0.250

** Master Gel; **Match Rate-1: The percentage of matched spots relative to the total number of spots on the gel;***Match Rate-2: The percentage of matched spots on the gel relative to the total number of spots on the master.*

Spot quantity can be calculated by PDQuest, which is the total intensity of a defined spot in the gel image. This corresponds to the amount of protein in the actual spot in the gel and it is calculated automatically during spot detection (11). The spot quantitation graphs are shown in Table 4 with protein spots number. The protein spots numbered 4302, 5401, and 8201 were detected in all gels of both groups and protein spots numbered 6301 and 6302 were observed in all gels of ER group while none of the gels of the OE group. The protein spots numbered 1401 and 4201 were observed in all gels of the OE group while only one gel of ER group. The locations of these protein spots on the gels were reported in Fig. 4.



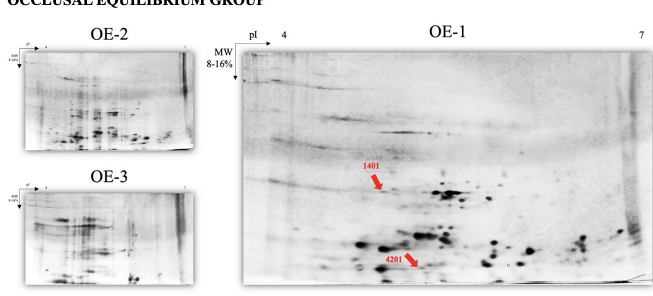


Figure 4. *Visual images of silver-stained gels. Protein spots that were analyzed with MS were marked.*

3.5. Identification of Proteins with Mass Spectrometry

Seven spots, four of which were differentially expressed between two groups and three of which were similarly expressed on both six gels, were selected for mass spectrometry analysis. After the in-gel digestion protocol, peptides were analyzed by mass spectrometry. A total of 7 proteins were identified from the spot samples, particularly keratins, albumin, and apolipoprotein A1 (Table 4).

Table 4. *Commonly and differentially expressed proteins between ER and OE groups and their quantitation graphs. Trypsin hits were omitted.*

Num. of spot and quantitation graph	Accession	Description	Mass	Score	Num. of significant matches
#4302	K1C10_HUMAN	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6	58792	74	1
	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	63	1
#5401	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	621	20
	K1C10_HUMAN	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6	58792	275	9
	APOA1_HUMAN	Apolipoprotein A-I OS=Homo sapiens OX=9606 GN=APOA1 PE=1 SV=1	30759	248	6
#8201	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	101	3
#6301	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	580	18
	K22E_HUMAN	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens OX=9606 GN=KRT2 PE=1 SV=2	65393	305	9
	K1C10_HUMAN	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN=KRT10 PE=1 SV=6	58792	507	12
	ALBU_HUMAN	Albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 SV=2	69321	52	1
#6302	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	341	13
	ALBU_HUMAN	Albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 SV=2	69321	55	1
#1401	K2C4_HUMAN	Keratin, type II cytoskeletal 4 OS=Homo sapiens OX=9606 GN=KRT4 PE=1 SV=5	56109	84	2
	K1C9_HUMAN	Keratin, type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN=KRT9 PE=1 SV=3	62027	65	1
#4201	K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN=KRT1 PE=1 SV=6	65999	165	5

4. DISCUSSION

Over the last century, various theories have been presented to understand the mechanisms of tooth eruption, but still, the mechanism behind this process is not yet fully unraveled (12). It has been shown that the tooth follicle regulates osteogenesis and osteoclastogenesis by secreting many different chemical mediators and plays a major role, particularly in the intraosseous phase of tooth eruption (13-18). While the dental follicle is necessary for eruption, it transforms into the periodontal ligament, once the tooth emerges from the gingiva (17). It is thought that extracellular matrix components such as collagen and fibronectin in the periodontal ligaments ensure tooth movement by creating tension and compression during the eruption (19). Several studies suggest that biochemical changes in GCF seem to reflect changes in the periodontal ligament (20, 21) and collection of this fluid is easy and non-invasive. Based on that

information, we designed this study considering that the biomarkers related to the supraosseous phase of the tooth eruption can be observed in GCF.

Since GCF is mostly in the field of periodontology and the gingiva gives serious reactions to more inflammatory conditions, comparative proteomic studies in GCF have been carried out mostly among adult patients of periodontal health and disease states (5, 7, 8, 22-26). Very few proteomic studies on GCF were conducted in pediatric samples (9, 27-29). In these studies, GCF samples were collected and analyzed with mass spectrometry-based tools to be able to demonstrate the differences in GCF composition in permanent and primary teeth, root resorption status, and pubertal growth states (9, 27-29).

When the tooth emerges into the oral cavity, the tooth movement rate is accelerated until the tooth reaches its occlusal contact. The post-emergent eruptive spurt phase, in which the fastest tooth movement occurs in the oral cavity, is thought to be worth investigating. While planning the study groups, mandibular permanent first molars were preferred for sampling. The most important reason for choosing it is that permanent molars are not succedaneous which means they do not replace any primary teeth. The presence of a shedding primary tooth while the permanent tooth is erupting may cause damage to gingival tissues, the gingiva may bleed, and the GCF content would be altered because of the exfoliated primary tooth. Therefore, the change between the post-emergent eruptive spurt phase and the occlusal equilibrium phase was examined in permanent first molars (2).

Due to the limited amount of GCF samples and their protein harvest, our first approach was to enrich the protein extraction output from GCF samples. Therefore, we compared two separate extraction solutions, urea buffer and rehydration buffer for their protein yield. Rehydration buffer provided more versatile protein bands, while urea buffer demonstrated only a few but dense protein bands when samples were separated on one-dimensional SDS polyacrylamide gel (Fig. 3a). For the next steps, we employed the rehydration buffer to extract proteins from GCF samples to examine the possibility of a wide range of proteins involved in the tooth eruption. We tested our protein extraction buffer efficiency with an additional clean-up step including Zeba™ Spin column to avoid any interference that might affect the resolution of two-dimensional polyacrylamide gels (Fig. 3b). When compared to the method in the study of Tsuchida et al (25), conventional urea buffer combined with ultrafiltration as the extraction method, our optimized protocol demonstrated higher resolution images of more protein spots which were not occluded due to the background noise or vertical streaks. However, the selection of the wide pI range for the IPG strip (3 to 10) caused spot overlapping due to similar molecular weight and a pI range of 5 to 6 for the GCF proteins extracted. For this reason, IPG strips of pH 4 to 7 were employed for the rest of the study. In the second dimension, corresponding GCF protein samples were separated with 8-16% gels followed by visualization with ProteoSilver™ silver stain kit.

When triplicates of 2D gel images of sample groups were compared, more protein spots were visualized in the OE group (OE, avg. 89; ER, avg. 67.6) according to PDQuest, suggesting that protein diversity and/or the number of protein modifications were higher in the OE group (Table 3). Later, we selected the spots displaying different intensities for in-gel trypsin digestion and mass spectrometry analyses to identify their protein contents (Fig. 4). We received hits for keratin type I cytoskeletal 10 (K1C10), keratin type II cytoskeletal 1 (K2C1), apolipoprotein A-1 proteins in spots number 4302, 5401 and 8201, and the proteins seen in these spots were detected in all gels of both groups (Table 4). The proteins we identified in 6301 and 6302 numbered spots were keratin type II cytoskeletal 1 (K2C1), keratin type II cytoskeletal 2 epidermal (K22E), keratin type I cytoskeletal 10 (K1C10) and albumin (Table 4). These spots were displayed only in ER group gels. We identified keratin type II cytoskeletal 4 (K2C4), keratin type I cytoskeletal 9 (K1C9), and keratin type II cytoskeletal 1 (K2C1) in spots numbered 1401 and 4201, and the intensity of these spots were lower in the ER group (Table 4). We encountered K1C9, K1C10, K2C1, K2C4, and K22E in our GCF samples due to the known expression of different types of cytokeratins in gingival pockets (7). Cytokeratins, which are cytoskeleton structural proteins, form intermediate filaments and are considered reliable markers of development and differentiation in epithelial cells (28).

The gingival sulcus is composed of oral sulcular epithelium and junctional epithelium. These components are a barrier against bacterial penetration and have a high turnover rate that allows rapid replacement of damaged cells and tissues (30). The normal turnover rate in healthy periodontium is known to be one of the most rapid of all epithelial tissues, and the keratin content in the crevice would be expected to be significantly greater than in other physiological fluids (30). In this study, among the keratins, K1C10 and K2C1 types were seen in similar amounts in both groups, while K2C4 and K1C9 type keratins were detected very rarely in the ER group but were found in the OE group. Elevation of the K2C4 and K1C9 proteins in GCF may be associated with the maturation of gingival tissue and permanent molar or with increasing age.

Bostanci et al (7) identified 25 different keratins and showed K1C9, K1C10, K2C1, and K2C4 were less regulated in aggressive periodontitis compared to healthy adult subjects. We also mention that K2C4 and K1C9 proteins are suppressed in tooth eruption, which might suggest a similarity in these two specific keratin types between tooth eruption and periodontal disease. When the tooth penetrates the oral mucosa, periodontal destruction occurs until the tooth crown fully emerges, and the gingival tissues give a physiological inflammatory response (9). On the other hand, Huynh et al (31) compared K1C9, K1C10, K2C1, and K22E levels in healthy, gingivitis, and chronic periodontitis adult subjects, and reported that the level of these proteins

was similar in healthy and gingivitis groups while elevated during periodontitis.

One of the most abundant proteins in GCF is albumin, which takes part in transport by binding to hormones, cytokines, and lipoproteins (7). Albumin was detected in pellicle and saliva, as well as in GCF samples (32). Bickel et al (33) and Carneiro et al (5) evaluated the albumin levels of GCF from healthy and periodontal disease adult subjects and reported that albumin levels were higher in periodontal disease groups. In this study, we observed that albumin levels increased in the eruption group as well. If the eruption of the tooth is considered a physiological inflammation status, albumin levels in GCF may indicate similar reactions just as in periodontal disease. When the protein profiles of pubertal subjects were compared to the post-pubertal, serum albumin was found to be higher in the pubertal group (29). The albumin levels in our GCF samples from the younger group demonstrate advanced intensities while it gets lower through ageing, which may imply the age-association effect on the albumin content of GCF (Table 4).

Apolipoprotein A-1 (ApoA-1) is the major protein component of high-density lipoprotein (HDL) (34). It was detected in both groups demonstrating no significant difference, similar to the studies identify ApoA-1 in their analysis (Table 4) (8, 23, 25, 29, 35). Only Moriya et al (28) reported that the ApoA-1 level was upregulated in the GCF of the permanent teeth compared to the primary teeth.

The periodontium is very vascular and highly permeable, therefore a variety of biomolecules in periodontal tissues might infiltrate the gingival sulcus making GCF an important biomarker. In this study, we tried to identify the GCF protein expression changes in two different phases of supraosseous tooth eruption. Proteins showing similarities and differences between these groups were determined. Overall, we report that the ER group displays slight similarities to the protein characterization in periodontal disease states (5, 7, 33). When the tooth first emerges from the gingiva, there might be a temporary physiological inflammatory response similar to that in periodontal disease, but not pathological. This study, which is the first GCF proteome study on tooth eruption, can be seen as a new way to look for the unknown components of this process. Although there are some limitations to conducting a proteomic study on GCF samples, such a relatively small sample volume with limited protein output and masking of low-concentration proteins by high-abundance proteins (31), it would still be manageable by optimizing protein extraction along with the application of new combinations of proteases to enrich peptide composition for mass spectrometry to identify more of the proteins. Our findings from this study support that GCF holds great potential to unravel the biochemical events relating to physiological and pathological conditions.

5. CONCLUSION

We present a mass spectrometry-based proteomics approach for the analyses of GCF, and we report several proteins related to tooth eruption. The novel protein extraction method for GCF resulted in better resolution for 2D-PAGE and image quantitation, applicable for future studies.

Funding: *This study was funded by Marmara University Scientific Research Projects Commission (Project-No: SAG-C-DRP-080.519.0181).*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Marmara University Faculty of Dentistry Clinical Research Ethics Committee (Approval date and number 2019-271).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: SYA, SA, AY, FS, HC

Design of the study: SYA, SA, AY, HÇ

Acquisition of data for the study: SYA

Analysis of data for the study: SYA, HC

Interpretation of data for the study: SYA, HC

Drafting the manuscript: SYA, SA, AY, HC

Revising it critically for important intellectual content: SYA, SA, AY, HC

Final approval of the version to be published: HC

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How to cite this article: Yıldırım Albat S, Akyüz S, Yarat A, Şahin F, Çimen H. Proteomic Analysis of Gingival Crevicular Fluid During Tooth Eruption. Clin Exp Health Sci 2023; 13: 863-870. DOI: 10.33808/clinexphealthsci.1214974