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ORIGINAL ARTICLE

Gingival crevicular fluid and salivary resistin and tumor necrosis factor-alpha levels in obese children with gingivitis

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Abstract

Background: This study aimed to evaluate the levels of resistin and tumor necrosis factor-alpha (TNF-α) in gingival crevicular fluid (GCF) and saliva of obese children with gingivitis.

Methods: One-hundred and thirty children (65 obese and 65 normal weight; age range 8 to 12 years) were recruited for the study. The children were classified into four subgroups based on their body mass and periodontal status; 1) obese children with gingivitis (OG, $n = 33$); 2) obese children with healthy periodontium (OH, $n = 32$); 3) normal weight children with gingivitis (NWG, $n = 32$); 4) normal weight children with healthy periodontium (NWH, $n = 33$). Body mass index (BMI) percentile, probing pocket depth (PPD), gingival index (GI), and plaque index (PI) were recorded. Resistin and TNF-α were analyzed in GCF and saliva samples by ELISA.

Results: Obese children had higher BMI percentiles than normal weight children ($p < 0.0001$). PPD, GI, PI, GCF volume, GCF, and salivary resistin and TNF-α levels were similar between obese and normal weight children ($P > 0.05$). OG and NWG subgroups had significantly higher GI, PI, GCF volume, GCF resistin total amounts, and salivary resistin concentrations but lower GCF resistin and TNF-α concentrations than OH and NWH ($P < 0.0001$ for all). GCF resistin total amounts were positively correlated with GI, PI, and GCF TNF-α total amounts ($P < 0.05$).

Conclusions: To our knowledge, this is the first study evaluated the levels of resistin in GCF and saliva of children. Obesity is not associated with GCF and salivary resistin and TNF-α levels in children in the presence of gingival inflammation.

KEYWORDS

cytokine(s), gingival crevicular fluid, gingivitis, obesity, saliva

1 | INTRODUCTION

Obesity and childhood obesity are among major public health concerns worldwide.[1] Obesity is considered as a chronic low-grade inflammatory process,[2] characterized by alterations in the systemic concentrations of some adipokines and adipocytokines[3] which also correlates with insulin resistance and the metabolic syndrome.[1–3]

Periodontal disease is caused by the immunoinflammatory response of the host against subgingival pathogenic

bacteria.[4] There is emerging evidence linking obesity with periodontal disease,[5,6] however the causal pathways are not fully understood. Adipose tissue is a metabolically active organ secreting adipokines and adipocytokines such as leptin, adiponectin, resistin, tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6[2,3], which function as proinflammatory and anti-inflammatory cytokines in periodontal tissue breakdown.[6,7] Moreover, obesity may impair the T-cell-mediated immune response[3] and increase the host's susceptibility to periodontitis.[8] Obesity-related chronic diseases such as type 2 diabetes mellitus, coronary heart disease, and metabolic syndrome could also worsen periodontitis.[7–9] A quantitative systematic review[10] showed TNF-α, IL-1β, IL-8, and resistin to be significantly higher in obese chronic periodontitis patients when compared to nonobese patients with periodontal disaese.

TNF-α seems to be a strong candidate linking obesity with periodontal disease.[6–9] Increased levels of TNF-α are a well-known risk factor for the onset of destructive periodontal disease.[11,12] It leads to the destruction of connective tissue and alveolar bone by enhancing both the secretion of matrix metalloproteinases and osteoclast formation.[12] Genco et al.[9] concluded that obesity is associated with high plasma levels of TNF-α and its soluble receptors, which in turn may lead to a hyperinflammatory state increasing the risk for periodontal disease. Previous studies reported obesity and metabolic syndrome were associated with elevated levels of GCF TNF-α of obese children and adolescents.[13–16]

Resistin, an adipokine, is mainly secreted by visseral white adipose tissue macrophages and peripheral blood mononuclear cells in humans, suggesting a link between resistin and inflammation.[17] Resistin acts as a proinflammatory molecule and stimulates the synthesis and secretion of TNF-α, IL-6, IL-12 via NF-kB pathway.[18,19] Resistin mRNA was strongly increased by TNF-α in human peripheral blood mononuclear cells.[20] Increased serum and GCF resistin levels were reported in chronic periodontitis patients when compared with periodontally healthy controls.[21,22] Many clinical reports documented that hyperresistinemia is associated with obesity-related chronic disorders like type 2 diabetes mellitus and atherosclerosis.[17] Therefore, resistin could be a potential biomarker for connecting obesity and periodontal disease. It has been claimed that obesity upregulates resistin secretion in GCF of patients with chronic periodontitis.[23,24]

Although several studies[25–28] have reported a positive relation between childhood obesity and gingival inflammation, some studies[29–31] demonstrated a null association in children. There were conflicting results and sparse pediatric data about underlying biological mechanisms between excessive body weight and gingival inflammation. To date, GCF and salivary resistin levels of children with gingivitis remains a question. Therefore, this study aimed to investigate whether obesity is associated with GCF and salivary resistin and TNF-α levels in children in the presence of gingival inflammation.

2 | MATERIALS AND METHODS

2.1 | Study population and clinical examination

One-hundred and thirty children (65 obese and 65 normal weight, age range 8 to 12 years) were included in this study at the clinics of Pediatric Dentistry Department, School of Dentistry, Adnan Menderes University, Aydın, Turkey, between January and August 2016. Ethical clearance was obtained from the local medical ethics committee (reference for approval 2015/37). Study details were explained in detail and written informed consent statement was obtained from the children's parents. Complete medical and dental histories were compiled for all participants, and followed by an oral examination.

The children were classified into four subgroups based on their weight status and periodontal conditions; 1) obese children with gingivitis (OG, $n = 33$); 2) obese children with healthy periodontium (OH, $n = 32$); 3) normal weight children with gingivitis (NWG, $n = 32$); 4) normal weight children with healthy periodontium (NWH, $n = 33$). Inclusion criteria were as follows: 1) aged between 8 and 12, 2) having the sex- and age-specific body mass index (BMI) between 5th and 85th percentile for normal weight children, 3) BMI ≥95th percentile for obese children, 3) being under the diet-control, 4) having fully erupted caries-free maxillary and mandibular incisors and first molars and exclusion criteria: 1) having any other known systemic diseases, 2) a diagnosed endocrine and hormonal effect that can cause obesity, 3) taking antimicrobial, anti-inflammatory and immunosuppressive drugs within the past 6 months, 4) having any destructive periodontal disease or periodontal therapy in the past 6 months, 5) having a restorative and endodontic therapy requirement, 6) orthodontic appliances, and 7) girls had menarche.

Obesity diagnosis was assigned according to the World Health Organisation growth reference data, international body mass index (BMI) cut offs for child overweight and obesity.[32] BMI was calculated as weight in kilograms divided by height in meters squared. Height was measured using a counter recording instrument (Harpenden Stadiometer, Holtain, Istanbul) and weight was measured using a digital medical weighing scale (Weighing scale, SECA 769, Istanbul). The weight of each participant was measured with light clothing and without shoes and bulky clothes. Both height and weight were measured twice and the averages were used for calculation. Because, in growing children BMI varies with age and sex, the BMI value was matched to a corresponding percentile on

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the international charts according to the patient's age and gender to calculate the BMI SDS (standard deviation score of patient's body mass index), which is based on accepted cut-off points. Sex and age-specific BMI percentiles were categorized into "normal weight" (< 85th percentile) or "obese" (≥95th percentile).

Clinical periodontal measurements including probing pocket depth (PPD), gingival index (GI)[33] and plaque index (PI)[34] were recorded at 12 fully erupted caries-free permanent teeth (maxillary and mandibular incisors and first molars) for representing whole mouth.[30] For each tooth, data were collected from four surfaces: buccal, lingual and palatinal, mesial, and distal. All clinical periodontal examinations were performed by a single calibrated pediatric dentist (GD) using a manual periodontal probe (Williams, Hu-Friedy, Chicago, IL). Calibration exercises for PPD and GI were performed in five children with gingivitis (aged between 10 and 12 years) before initiaton of the study. Each children was assessed twice during one visit over a 1-hour interval. Intraexaminer reliability was 0.87 for PPD and 0.85 for GI (values calculated by Cohen k-test). PPD was measured as the distance from the gingival margin to the bottom of the probed pocket. The presence of marginal gingival bleeding and the amount of visible plaque in accordance with the index systems was recorded.[33,34] Gingivitis was diagnosed when GI ≥1, PPD ≤3 mm at all measured sites for 12 permanent teeth. Children were accepted as periodontally healthy if they had GI < 1 and PPD < 3 mm for these teeth. Because none of the patients had clinical attachment loss, clinical attachment level was not calculated. The number of the permanent teeth, deciduous teeth, and total teeth number were recorded for each participant.

2.2 | Saliva and GCF sampling

To diminish the influence of circadian rhythm on biomarker levels, all samples were obtained in the morning between 8:00 am and 10:00 am. One day after periodontal clinical measurements, the whole unstimulated saliva samples were collected according to a modification of the method described by Navazesh.[35] The participants were instructed to refrain from oral hygiene practices including flossing, brushing, and mouth-rinses as well as eating, and drinking for 2 hours before collection. Before saliva collection, each participant was requested first to rinse the mouth completely with water for 2 minutes, wait for 10 minutes, and then, for 5 minutes, expectorate into sterile 50-mL polypropylene tubes. Saliva samples were then placed on ice, aliquoted and frozen at -80°C, until the analysis. On the day of analysis, samples were thawed on ice, centrifuged at 10,000 × *g* for 15 minutes at 4°C and immediately used for further analysis.

The GCF samples were obtained from four nonadjacent sites at fully erupted caries-free permanent maxillary incisors. Samples were taken from the mesio-buccal and disto-buccal sites with visible plaque accumulation and exhibiting severe inflammation with a GI score of 2 and 3 for gingivitis groups. GCF was sampled with filter paper strips (PerioPaper, Proflow, Amityville, NY). Supragingival plaque was removed without touching the marginal gingiva by sterile curettes, the crevicular site was gently dried with an air syringe and isolated with a cotton roll. A sterile paper strip was gently inserted into the crevice until mild resistance was felt and left in place for 30 seconds. GCF volume was measured with a precalibrated electronic device (Periotron 8010, Oraflow, Amityville, NY). Care was taken to avoid mechanical injury to periodontal tissue. Strips contaminated with blood were discarded. The four strips from each patient were placed into polypropylene tubes separately and frozen. All strips were stored at −80°C until further processing.

2.3 | Measurement of resistin and TNF-α levels in GCF and saliva

Four paper strips were pooled, placed in 350 μL PBS-T (0.05%) and incubated for 20 minutes at room temperature on an orbital shaker (240 rpm). The fluid from the paper strip was recovered by centrifugation at 13,000 rpm for 5 minutes at + 4°C and stored at -20°C. Frozen saliva samples were thawed, preheated up to 37°C and mixed thoroughly before analysis. Resistin and TNF-α levels in GCF and saliva samples were measured by the enzyme-linked immunosorbent assay (ELISA) using commercial kits (Human Resistin ELISA kit, Sunred Biotechnology, Shanghai and Human Tumor necrosis factor-α ELISA kit, Sunred Biotechnology, Shanghai) according to the manufacturer's guidelines. The minimum detection limits for resistin and TNF-α kits were 0.25 ng/mL and 2.827 ng/L respectively. Plates were measured at 450 nm with 650 nm as a reference wavelength by using an ELISA reader (DTX 880 Multimode Reader, Beckman Coulter, Miami, FL). Cytokine concentrations were calculated from the standard curve.

Data for GCF were reported as total amount of cytokines in nanograms per sample (ng/sample) and concentrations of cytokines in nanograms per milliliter (ng/μL). Before analysis, GCF was eluted from pooled four paper strips with 350 μL PBS-T (0.05%). After running the assay, resistin levels were detected as ng/ml and TNF-α levels were detected as ng/L. Total amount in 350 μL PBS-T for both cytokines was calculated as ng. GCF volume was calulated as μL. Then, concentrations of both cytokines in GCF were calculated by dividing total amount (ng) by the volume of GCF (μL). Resistin and TNF-α concentration in saliva were detected as ng/mL and ng/L, repectively.

2.4 | Statistical analysis

Minimum sample size was calculated using a specialized software package for power analysis (G*Power version 3.0.8,

TABLE 1 Anthropometric data and periodontal clinical parameters of obese and normal weight children

Parameters/Groups	Normal weight (n: 65)	Obese (n: 65)	Body mass effect *p* value
BMI (kg/m^2)	17.78 (15.31 – 20.40)	26.30 (20.71 – 35.61)	**<0001**
BMI percentile (%)	49.23 (3.67 – 78.84)	97.34 (95.00 – 99.63)	**<0001**
BMI SDS	−0.02 (-1.79 – 0.80)	1.93 (1.37 – 2.68)	**<0001**
PPD (mm)	1.41 (0.93 – 2.26)	1.37 (0.98 – 2.19)	0.357
GI	0.18 (0 – 2.41)	1.10 (0 – 2.10)	0.564
PI	0.96 (0.04 – 2.75)	1.10 (0.02 – 2.37)	0.707
GCF volume (μl)	0.13 (0.03 – 1.10)	0.13 (0.01 – 0.48)	0.891

All data are shown as median (q1-q3). The significant differences level of $p < 0.05$ are shown in bold face.

Heinrich Heine University, Düsseldorf). Any dependent variable in the 2 × 2 factorial ANOVA Model, with a power of 80%, 0.25 f-type effect size, and an $\alpha = 0.05$, the minimum number of patients required for each group was 32. To compensate probable missing sample or data, the sample size was adjusted to 130.

Descriptive statistics and normality test of obese and normal weight subgroups were performed using a statistical software package (SPSS v22.0, IBM, Chicago, IL). The distribution of the clinical and biochemical data was validated by Shapiro Wilk's normality test in each subgroup which included 32 or 33 children (samples size < 50). Because the majority of the variables in the statistical analyses did not show a normal distribution, the analyses were performed using nonparametric methods. For multiple group comparisons, nonparametric 2 × 2 factorial analysis was performed with a software program (SAS 9.3 Software, SAS Institute Inc.,Cary, NC). Effect of body mass, periodontal status, and body mass × periodontal status interaction on all clinical and biochemical variables were tested with the nonparametric model of Brunner and Langer. The categorical variables were analyzed by chi-square analysis, the linear relationship between clinical and biochemical variables examined using Spearman's Rank Correlation Analysis. All the tests were performed at $\alpha = 0.05$.

3 | RESULTS

The mean age (years ± SD) were 10.60 ± 1.20 for obese children and 10.98 ± 1.02 for normal weight children. The sex distribution (female/male) were 25/40 and 27/38 for obese and normal weight children, respectively. There were no statistically significant differences between the obese and normal weight children for sex and age ($P > 0.05$ for both).

Anthropometric data and periodontal clinical parameters of obese and normal weight children are presented in Table 1. BMI, BMI percentile, and BMI SDS values in obese children were significantly higher than normal weight children ($P < 0.0001$ for all). The number of the permanent teeth, deciduous teeth, and total teeth number were similar between two groups ($P > 0.05$) (data not shown). PPD, GI, and PI values of 12 permanent teeth and GCF volume in obese children were similar to normal weight children ($P > 0.05$). Resistin and TNF-α were detected in all samples. There were no significant differences in GCF resistin and TNF-α total amounts between obese and normal weight children ($P > 0.05$) (Figure 1A, Figure 1B) Resistin and TNF-α concentrations in both GCF and saliva were similar between two groups ($P > 0.05$) (Figures 1C through 1F).

Anthropometric data and periodontal clinical parameters of obese and normal weight subgroups are presented in Table 2. BMI, BMI percentile, and BMI SDS values in OG and OH subgroups were significantly higher than NWG and NWH subgroups ($P < 0.0001$ for all). PPD scores of 12 permanent teeth were similar among subgroups ($P > 0.05$). OG and NWG subgroups had significantly higher GI and PI scores for these teeth compared to OH and NWH subgroups ($P < 0.0001$ for both). Although PPD scores of sample sites were similar among subgroups ($P > 0.05$), GI and PI scores in gingivitis subgroups were higher than periodontally healthy subgroups ($P < 0.0001$ for both) (data not shown).

The results of biochemical analyses in GCF and salivary samples of obese and normal weight subgroups are outlined in Figure 2. OG and NWG subgroups had significantly higher GCF resistin total amounts and salivary resistin concentrations than OH and NWH subgroups ($P < 0.0001$ for both) (Figures 2A and 2E). GCF resistin and TNF-α concentrations in OG and NWG subgroups were significantly lower than periodontally healthy subgroups ($P < 0.0001$ for both) (Figures 2C and 2D). GCF TNF-α total amounts and salivary TNF-α concentrations were similar among four subgroups ($P > 0.05$) (Figures 2B and 2F).

Correlations between clinical periodontal parameters and biochemical data are demonstrated in Table 3. GCF resistin total amounts were positively correlated with PI, GI, and GCF TNF-α total amounts ($r = 0.396$, $P = 0.01$; $r = 0.359$, $P = 0.01$; $r = 0.314$, $P = 0.01$, respectively). A positive correlation was observed between resistin and TNF-α concentrations in saliva ($r = 0.567$, $P = 0.01$).

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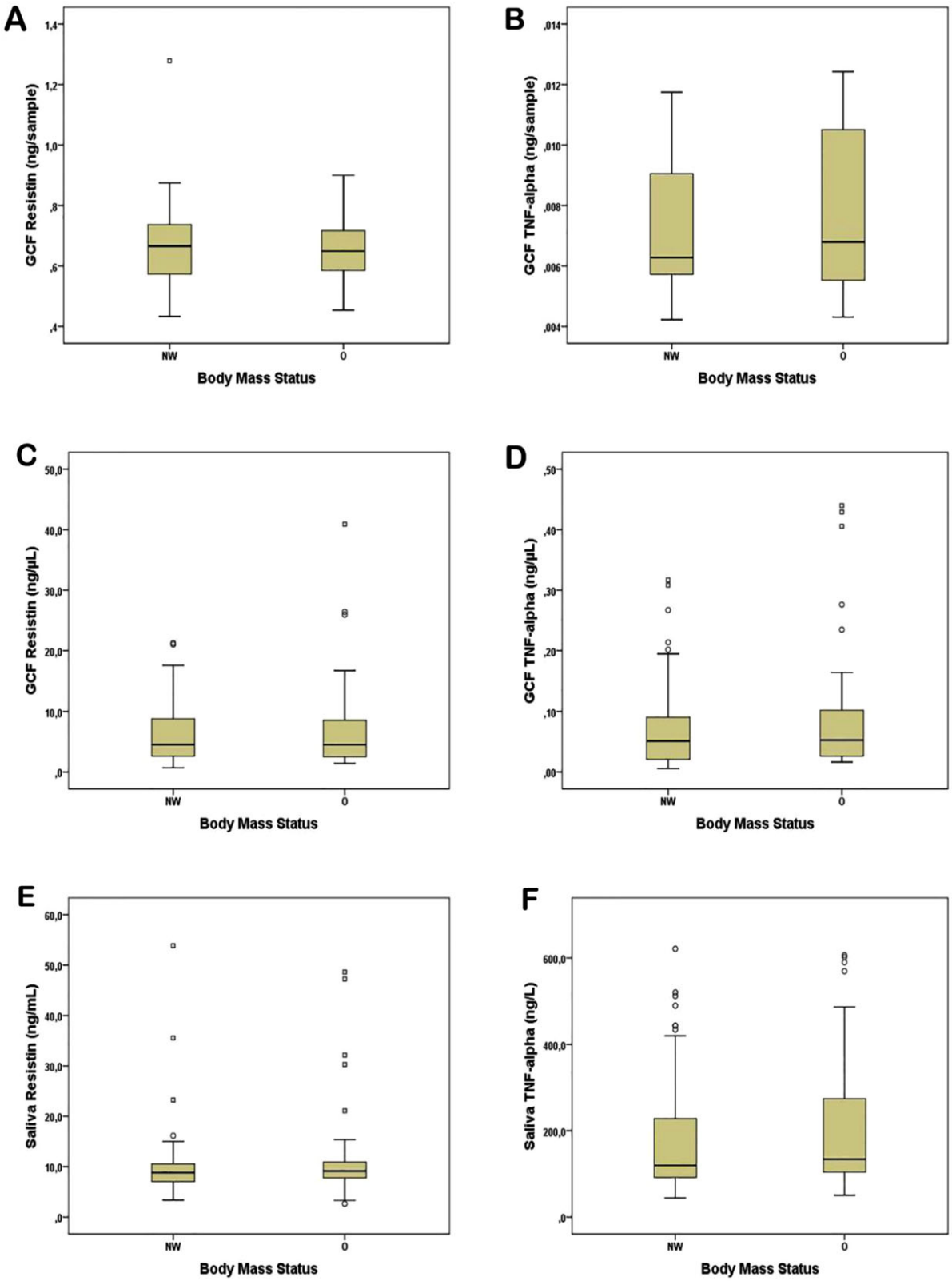

FIGURE 1 Biochemical data of obese (O) and normal weight (NW) groups. A) GCF resistin total amount. B) GCF TNF-α total amount, C) GCF resistin concentration, D) GCF TNF-α concentration, E) Saliva resistin concentration, F) Saliva TNF-α concentration. The solid horizontal lines indicate median values

TABLE 2 Anthropometric data and periodontal clinical parameters of obese and normal weight children

Parameters/ Groups	Normal Weight		Obese		Periodontal status effect p value	Body mass* periodontal status interaction p value
	NWH (n:33)	NWG (n:32)	OH (n:32)	OG (n:33)		
BMI (kg/m^2)	17.79 (15.31–20.34)	17.78 (15.75–20.40)	26.51 (20.71–32.89)	26.14 (21.89–35.61)	0.460	0.573
BMI percentile (%)	48.57 (3.67–78.84)	51.17 (11.37–77.34)	97.48 (95.00–99.22)	97.22 (95.00–99.63)	0.557	0.981
BMI SDS	−0.04 (−1.79–0.80)	0.03 (−1.79–0.80)	1.95 (1.37–2.42)	1.91 (1.38–2.68)	0.675	0.871
PPD (mm)	1.50 (0.93–2.00)	1.41 (1.12–2.26)	1.23 (0.98–2.00)	1.40 (1.00–2.19)	0.052	0.357
GI	0.08(0.00–0.01)	1.71* (1.10–2.41)	0.07 (0.00–0.16)	1.61† (1.10–2.10)	**<0001**	0.552
PI	0.37 (0.04–1.04)	1.64* (0.96–2.75)	0.30 (0.02–0.98)	1.61† (1.10–2.37)	**<0001**	0.735
GCF volume (μl)	0.06 (0.03–0.13)	0.28* (0.12–1.10)	0.07 (0.01–0.13)	0.26† (0.02–0.48)	**<0001**	0.149

All data are shown as median (q1-q3). The significant differences level of $p < 0.05$ are shown in bold face.

*NWG significantly different than NWH and OH

†OG significantly different than OH and NWH

4 | DISCUSSION

To the best of our knowledge, this is the first study to evaluate the levels of resistin and TNF-α in GCF and saliva of obese and normal weight children with or without gingivitis. GCF and salivary resistin levels increased in the presence of gingival inflammation in both obese and normal weight children, and the GCF resistin total amount was positively correlated with GI and PI.

Plaque-induced gingivitis is a highly prevalent form of peridontal disease among children and adolescents.[36,37] Obesity-related hyperinflammatory state and altered immune cell activity may provoke the inflammatory response to plaque and the microbial challenge in periodontal tissues.[7–10] Based on this hypothesis, several studies[25–28] have reported a positive relation between pediatric obesity and gingivitis by evaluating clinical periodontal parameters. BMI, the reliable indicator of body fat mass, was found to be positively associated with the gingival bleeding accepted as a stronger indicator of gingival inflammation. Obese children and adolescents had the higher PPD, PI, GI, and BOP values than normal weight counterparts.[25–28] It has been speculated that unhealthy dietary habits and poor oral hygiene attitude in obese children may lead to the higher plaque scores which is associated with the higher GI and BOP.[25–28] In contrast to aforementioned studies[25–28], our data revealed that obese children had similar PPD, PI, and GI values to normal weight children although they had higher BMI values as expected. Discrepancy among studies could be attributed to the differences in selected age groups, used clinical index systems, the teeth numbers and sites evaluated and the criteria used for the diagnosis of gingivitis. In the present study, PPD, plaque accumulation and marginal gingival bleeding were recorded at 12 permanent teeth. It was important to ensure the cooperation of children to achieve more sensitive and accurate measurements. These teeth were also selected to minimize the effect of the eruption gingivitis and also standardization of measured teeth in mixed dentition in selected age group.[30] Our findings are consistent with other studies[29–31] suggesting that the severity of gingival inflammation in children was not affected by obesity.

In the present study, OG and NWG subgroups had significantly higher GCF volume than periodontally healthy subgroups despite similar PPD scores. Diagnosis of gingivitis is mainly relied on the clinical findings and manifestations including redness and edema of the marginal gingiva and bleeding on probing.[36,37] GCF volumes are directly related to the severity of the gingival inflammation because of increased vascular permeability and ulceration of the epithelium at inflamed sites.[38] Griffiths et al.[39] showed that GCF volume were associated with the sign of gingival inflammation rather than any clinical periodontal measurements.

Resistin and TNF-α levels in GCF have been reported as total amount per sample as well as concentration in this study. However, the discussion was based mainly on the GCF total amounts of both cytokines. Because the GCF volumes are invariably low at healthy sites, there will be a tendency to overestimate the concentrations of cytokines in GCF,[40,41] which in turn can cause to a misleading evaluation about the role of cytokines in peridontal disease. As expected, GCF concentrations of resisitin and TNF-α in periodontally healthy subgroups were significantly higher than the gingivitis subgroups because of the low GCF volume.

Our data revealed that similar GCF and salivary resistin levels were found in obese and normal weight groups. In the literature, there is no study investigating the resistin levels in GCF and saliva of children, therefore we were not able totions to the study. This study compare our study with others. In adults, some studies suggested that obesity upregulates resistin secretion in GCF of patients with chronic periodontitis.[23,24] In contrast, Zimmerman et al.[42] and Patel and Raju[43] reported

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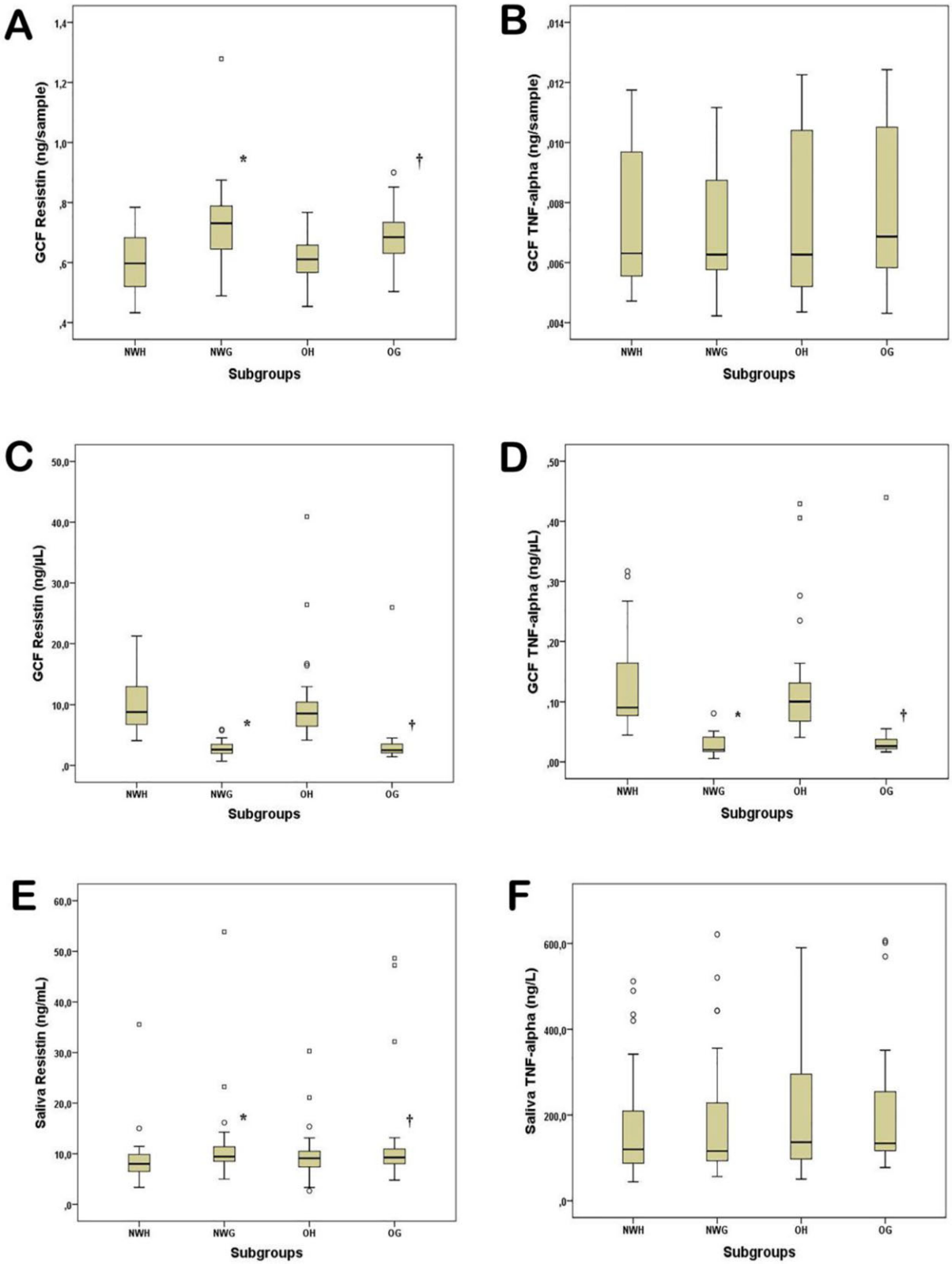

FIGURE 2 Biochemical data of the NWH, NWG, OH and OG subgroups. A) GCF resistin total amount. B) GCF TNF-α total amount, C) GCF resistin concentration, D) GCF TNF-α concentration, E) Saliva resistin concentration, F) Saliva TNF-α concentration. The solid horizontal lines indicate median values. Significant differences between groups are shown as follows: $^{*}p < 0.0001$, NWG significantly different than NWH and OH; $^{\dagger}p < 0.0001$, OG significantly different than OH and NWH

TABLE 3 Correlations between clinical parameters and biochemical findings

Parameters	PPD	GI	PI	GCF volume	GCF resistin	GCF TNF-α	Salivary resistin	Salivary TNF-α
BMI	−0.062	0.039	0.068	0.074	−0.045	0.109	0.007	0.071
GCF volume	0.061	0.791*	0.789*	1.000	0.427*	0.057	0.177†	0.082
GCF resistin	0.038	0.359*	0.396*	0.427*	1.000	0.314*	0.084	0.030
GCF TNF-α	0.051	0.052	0.021	0.057	0.314†	1.000	−0.012	0.023
Salivary resistin	0.064	0.047	0.110	0.177†	0.084	−0.012	1.000	0.567*
Salivary TNF-α	−0.124	0.001	0.041	0.082	0.030	0.023	0.567*	1.000

*Correlation is significant at the 0.01 level (2-tailed).
†Correlation is significant at the 0.05 level (2-tailed).

similar serum and GCF resistin levels in obese and nonobese chronic periodontitis patients. However, it should be kept in mind that the results of these adult studies may not be generalizable to children when considered the complex nature of obesity and periodontitis, which is characterized by alveolar bone resorption and clinical attachment loss.

One of the most important findings of the current study was that gingivitis subgroups had elevated GCF and salivary resistin levels compared to periodontally healthy subgroups. Previous studies in which patients with chronic periodontitis showed significantly higher GCF resistin levels when compared to periodontally healthy controls.[22,23,42–44] It is not surprising to find a strong, positive correlation between GCF resistin levels and clinical parameters of periodontal inflammation and proinflammatory cytokine TNF-α. Resistin is secreted by immunoinflammatory cells, most predominantly macrophages, and is related to the modulation of inflammatory responses.[17] The expression of resistin from neutrophils is regulated by periodontopathic bacteria.[22] It has been claimed that resistin is induced in response to various inflammatory stimuli such as lipopolysaccharide, TNF-α, or IL-6[20] and resistin itself induces proinflammatory cytokines via NF-kB pathway.[18,19] In the present study, increased resistin levels in GCF of children with gingivitis and its positive correlation with TNF-α could be attributed to the proinflammatory role of resistin in plaque-induced gingival inflammation rather than obesity-induced systemic inflammation. In other words, gingival inflammation might modulate the resistin levels, irrespective of obesity.

In contrast to our findings, previous studies have indicated that the GCF TNF-α levels were significantly elevated in obese children[13,14] and adolescents.[15] Lundin et al.[15] have shown that GCF TNF-α levels were correlated with BMI in the most severe obese adolescent (BMI ≥40) with no pathological periodontal pocket. Ka et al.[16] found a positive association between waist circumference and GCF TNF-α level in boys with metabolic syndrome including obesity. Because the serum levels of TNF-α were not evaluated in these studies,[13–16] it is not clear whether GCF TNF-α levels truly reflect the levels found in serum. However, in line with our findings, Modeer et al.[25] reported no significant differences in GCF TNF-α levels between periodontally healthy obese and nonobese adolescents. It can be speculated that the differences between studies can caused by the severity and duration of obesity.[15,25] In our study, the participants were children and they had lower BMI scores when compared to adolescents, indicating a shorter duration and less severe of obesity. Although we did not register the obesity duration, it is likely to be important in the initiation and progression of periodontal tissue breakdown.[25] In addition, Fell et al.[45] found no correlation between the GCF and serum levels of TNF-α from healthy and gingivitis sites in obese individuals. Considering the conflicting results, further studies need to clarify the origin of TNF-α in GCF of obese children.

It has been generally accepted that TNF-α, a potential marker of periodontal inflammation, were significantly elevated in GCF and gingival tissue biopsies of patients with periodontal disease.[11,12] It is surprising to note that GCF and salivary TNF-α levels in gingivitis groups did not differ from periodontally healthy groups in this study. Similar to our findings, Ülker et al.[46] found similar GCF TNF-α levels in children with gingivitis and periodontally healthy controls. As an interesting result, they found salivary TNF-α levels in periodontally healthy children were higher in children with gingivitis.[46] Gornowicz et al.[47] reported that elevated levels of salivary cytokines such as ILs and TNF-α were seen in children with dental caries. Therefore, we excluded the children who have a restorative and endodontic therapy requirement. Considering the complex nature of saliva, which comprises of a mixture of fluids containing proteins from the salivary glands, GCF, bacteria, mucous from the nasal cavity and pharynx,[48] it is reasonable to speculate that TNF-α and resistin levels in saliva of children can derive from different sources. In line with the results of our study, several studies in adults showed there were no relationship between the salivary concentration of TNF-α and periodontal inflammation.[49,50] Further studies with larger sample size are needed to evaluate the TNF-α levels in both GCF and saliva of children.

The present study has some important limitations that should be considered. Children's systemic condition were

recorded only by the receiving data from their parents. Therefore, it is not exactly clear whether they had any systemic disease other than obesity. We used BMI representing general adiposity, however it has been suggested that visceral adiposity is more related to inflammatory cytokine levels.[5] Adipometric measurements for visceral adiposity could best help to further highlight the biochemical connection between obesity and gingival inflammation. There is evidence that childhood obesity can induce early puberty.[28] Although care was taken to ensure that the children were in the prepubertal stage in this study, obese children might have been in the peripubertal period. Hence, our findings may have been influenced by the covert hormonal activation.

5 | CONCLUSIONS

Considering the limitations of the matched case-control study design, the present findings suggest that childhood obesity is not associated with GCF and salivary resistin and TNF-α levels. On the other hand, the presence of gingivitis, irrespective of body mass, seems to have a pronounced effect on resistin levels in both GCF and saliva of children. Therefore, the potential role of resistin in gingival inflammation with or without obesity deserves further large-scale trials and follow-up investigations.

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