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# DNA damage and oxidative status in PFAPA syndrome

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ABSTRACT

*Objective:* PFAPA syndrome is a clinical entity of unknown etiology which presents with periodic episodes of fever, aphthous stomatitis, tonsillitis or pharyngitis, and cervical adenitis. In this study we investigated DNA damage and the oxidative stress parameters in patients diagnosed with PFAPA, to elucidate the underlying pathophysiological mechanism of this syndrome.
*Methods:* Thirty-one patients diagnosed with PFAPA (Group 1), 22 patients diagnosed with normal tonsillitis or pharyngitis (Group 2), and 20 healthy volunteers (Group 3) were included in our study. Heparinized peripheral blood samples were drawn from all patients and volunteers. DNA damage was assessed by single cell alkaline electrophoresis assay in peripheral mononuclear leukocytes. Plasma levels of total antioxidant status (TAS) and total oxidative status (TOS) were determined by using a novel automated measurement method, and oxidative stress index (OSI) was calculated.
*Results:* DNA damage in the mononuclear leukocytes of Group 1 was significantly higher than that of Group 2 and Group 3. The oxidative stress parameters revealed that the TOS and OSI values of Group 1 were significantly higher than those of Group 2 and Group 3. TAS values of Group 1 were significantly lower than those of Group 2 and Group 3. Correlation analysis of Group 1 demonstrated a significant correlation between TOS, one of the oxidative stress parameters, and DNA damage. Correlations between DNA damage and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values were also significant.
*Conclusion:* Our study indicated that both the inflammatory and the oxidative stress parameters were significantly increased in patients with PFAPA syndrome, accompanied by a significant positive correlation between DNA damage and oxidative stress.


## 1. Introduction

PFAPA syndrome, first described by Marshall et al. [1] in 1987 as a syndrome affecting the pediatric age group, is a clinical entity of unknown etiology, which consists of recurrent periodic fever, aphthous stomatitis, tonsillitis or pharyngitis, and cervical lymphadenopathy [2].

Periodic fever usually occurs regularly every 3–8 weeks, and the febrile state generally continues for 3–6 days. There may also be some other accompanying symptoms, such as nausea, vomiting, headache, abdominal pain, muscle pains, diarrhea, arthralgia, cough, coryza and rash [3]. Diagnosis is reached by the presence of the above clinical criteria, plus by the exclusion of other periodic fever syndromes [4]. In current clinical practice, the diagnosis of PFAPA follows the criteria proposed by Thomas et al., and achieved widespread acceptance [2]. Viral and autoimmune mechanisms are being considered in the etiology of PFAPA, but so far the exact cause remains unidentified [1,5]. Studies on PFAPA syndrome revealed an increase in proinflammatory cytokines and a decrease in anti-inflammatory cytokines (during attacks) [6]. Increased levels of TNF-α, IFN-γ and IL-6 during attacks are also in favor of an ongoing inflammatory process [1,7]. Considering the above information, we have designed this study based on the thought that this inflammatory process would generate an oxidative stress in the organism.

Oxidative stress (OS) is an indicator of the disturbed balance between the generation of reactive oxygen species (ROS) and the antioxidant mechanisms set forth to detoxify those free radicals. In

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normal physiological circumstances, ROS is not hazardous for the organism since it is balanced by antioxidants. When ROS concentrations exceed physiologic values, antioxidants like the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT), and other non-enzymatic molecules like glutathione, uric acid, melatonin, and vitamins A, C and E, cannot inhibit the potent cytotoxic effects of those free radicals, and the oxidative balance of the organism shifts toward oxidative stress. Antioxidants are used up in detoxifying oxidant molecules, and the resultant oxidative stress is characterized by a decreased amount of antioxidants [8]. The increased oxidative stress causes oxidative damage to certain macromolecules of the organism, namely the proteins, lipids and the DNA. Recent studies demonstrated a strong correlation between oxidative stress and DNA damage [9,10].

Various methods are being used for monitoring DNA damage in peripheral mononuclear leukocytes, which is an indicator of the overall DNA damage in the body. Micronucleus (MN) test, chromosomal aberration analysis, the sister-chromatid exchange (SCE) test, gene mutation tests, and the comet assay test can be cited among such methods [11]. The comet assay (single-cell electrophoresis) test is a simple, rapid and sensitive test, and has a widespread use for determining the extensiveness of endogenous DNA damage [11,12]. Total oxidative status (TOS) and total antioxidant status (TAS) are parameters used widely for assessing the oxidative balance. Since measuring various oxidant and antioxidant molecules one by one is not practical, those parameters are used instead, and determine the total oxidant and anti-oxidant status [13,14].

There are numerous studies in medical literature focused on elucidating the pathophysiological basis of PFAPA syndrome, while no research has yet investigated the oxidative balance and DNA damage in such cases. Our study aims to explore DNA damage and the oxidative balance in patients clinically diagnosed with PFAPA.

## 2. Materials and methods

### 2.1. Subjects

Our study was conducted during March 2013 and January 2014, after obtaining the approval of the Local Clinical Research Ethics Committee. This cross-sectional study included 31 patients who were diagnosed with PFAPA (Group 1: PFAPA) in our university (Ear Nose and Throat and Pediatrics Clinics), 22 patients diagnosed with normal tonsillitis or pharyngitis (Group 2: NTP), and 20 healthy volunteers with similar demographic profiles (Group 3: CNT). The study design complied with the Helsinki Declaration principles. The study design was explained to the parents of all patients and volunteers, and written consents were obtained from all. Participants with chronic disorders, acute bacterial infection or other autoimmune diseases were excluded.

Patients who complied with the diagnostic criteria proposed by Thomas et al. [2] and accepted by the international medical community were included in the patient group of the study (Table 1). Patients were followed up for at least three periods of febrile episodes. Their demographics, clinical information and laboratory data were recorded. Patients who had infections or diagnosed with any other periodic fever syndrome were excluded from the study. Blood samples were drawn during the febrile episodes.

Twenty-two patients who seek for medical advice from Ear, Nose and Throat Department clinics in our university were diagnosed with tonsillitis or pharyngitis, included to the Group 2. These patients had no aphthous stomatitis, periodic episodes of fever, and cervical adenitis.

They did not have any other systematic diseases.

**Table 1**
Diagnostic criteria of the PFAPA syndrome.

| Diagnostic criteria for Marshall's/PFAPA syndrome [2] |
|---|
| 1. Regularly recurring fevers with an early age of onset (<5 years of age) |
| 2. Symptoms in the absence of upper respiratory tract infection with at least one of the following clinical signs: |
| (a) Aphthous stomatitis |
| (b) Cervical lymphadenitis |
| (c) Pharyngitis |
| 3. Exclusion of cyclic neutropenia |
| 4. Completely asymptomatic interval between episodes |
| 5. Normal growth and development |

The control group (CNT) consisted of 20 healthy volunteers who did not have a history of PFAPA or a history of any other disease and/or drugs (during the past month). The volunteers were children who were being followed up by our University's Healthy Child Clinic, and were randomized from routine control appointments. Their demographics, clinical information and laboratory data were recorded. Blood samples were drawn from peripheral venous veins.

### 2.2. Blood collection and storage conditions

Five ml venous blood samples were drawn from the forearms of all participants. Blood samples were immediately drawn into heparinized tubes and were stored in dark, at a temperature of 2–4 °C, and were processed within 2 h to avoid any DNA damage due to hemolysis. Isolation of mononuclear leukocytes was conducted by centrifugation with Histopaque 1077 (Sigma). One ml of the heparinized blood specimen was carefully laid over Histopaque, and centrifuged at 500 × *g* for 35 min, at 25 °C. The interface band with the mononuclear leukocytes was centrifuged again with phosphate-buffered saline (PBS), at 400 × *g* for 15 min. Membrane integrity was measured with Trypan-Blue exclusion assay, and samples with over 90% cell viability were used for assessing DNA damage. The remaining heparinized blood specimens were centrifuged at 1500 × *g* for 10 min, and the plasma thus obtained was stored in −80 °C for the TOS and TAS analyses.

### 2.3. Determination of DNA damage using the alkaline comet assay

The comet assay, or the single-cell gel electrophoresis (SCGE) assay, was conducted with some modifications of the Singh et al.'s [11,15] method. Approximately 10 μl of fresh mononuclear leukocyte cell suspension (approx. 20,000 cells) was mixed with 80 μl of 0.7% low melting-point agarose in PBS at 37 °C. Next, 80 μl of each mixture was layered onto a slide precoated with a thin layer of 1% normal melting-point agarose (NMA) and immediately covered with a cover slip. Slides were held for 5 min at 4 °C to allow the agarose to solidify. After removal of cover slips, the slides were immersed in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, 1% Triton X-100, and 10% DMSO; pH 10–10.5) for at least 1 h. Triton X-100 and DMSO were added to the solution just before use. Slides were next immersed in freshly prepared alkaline electrophoresis buffer (0.3 M NaOH and 1 mM Na2EDTA; pH > 13) at 4 °C to allow DNA to unwind (40 min) and then electrophoresed (25 V/300 mA, 25 min). All manipulations were performed under minimal illumination. After electrophoresis, the slides were neutralized (0.4 M Tris–HCl; pH 7.5) for 5 min. Dried microscope slides were stained with ethidium bromide (2 mg/ml in distilled water; 70 μl/slide), covered with cover slips, and viewed by fluorescence microscopy (Olympus BX51, Japan) at 200x magnification. The microscope was capable of detecting epifluorescence and was equipped with a rhodamine filter (excitation wavelength 546 nm; barrier 580 nm).

The extent of extranuclear fluorescence was scored (by eye) in 50 random cells of each sample using a scale of 0–4 as previously described by Kobayashi et al. [16]. Scoring was as follows: 0, no tail; 1, comet tail < half the width of the nucleus; 2, comet tail equal to the width of the nucleus; 3, comet tail longer than the width of the nucleus but not twice as long; 4, comet tail > twice the width of the nucleus. This type of scoring has been shown to be as accurate that afforded by computerized image analysis [12]. All slides were coded and were scored in a blinded manner. A visual score for each class of subjects was calculated by multiplying the percentages of cells in the various comet classes by the score for that class. The total visual comet score reflecting the extent of DNA damage was the sum of scores for all five comet classes. Thus, a total visual score could range from 0 (all undamaged) to 400 (all maximally damaged) in arbitrary units (AU).

### 2.4. Measurement of total oxidant status

Plasma TOS was measured using a novel automated method developed by Erel [13]. Oxidants present in a sample oxidize the ferrous ion of an o-dianisidine complex to ferric ion. Oxidation is enhanced by glycerol, which is abundant in the reaction medium, and the ferric ion forms a colored complex with xylenol orange under acidic conditions. Color intensity (which can be measured spectrophotometrically) is associated with the total level of oxidants present. Hydrogen peroxide is used to calibrate the assay and results are expressed in terms of micromoles of hydrogen peroxide equivalent per liter (mmol $H_2O_2$ equiv./l).

### 2.5. Measurement of total antioxidant status

Plasma TAS was measured using another novel automated method developed by Erel [14]. This involves production of the hydroxyl radical, which is a potent biological reactant. A ferrous ion solution (Reagent 1) is mixed with hydrogen peroxide (Reagent 2). Radicals produced by the hydroxyl radical, including the brown dianisidinyl radical cation, are also potent in biological terms. Thus, it is possible to measure the antioxidative capacity of a sample in terms of inhibition of free radical reactions initiated by production of the hydroxyl radical. Variation in assay data is very low (less than 3%) and results are expressed as mmol Trolox equiv./l.

### 2.6. Measurement of oxidative stress index

The OSI was the TOS-to-TAS ratio, but TAS values were changed to mmol/l. Each OSI was calculated as follows: OSI (arbitrary units) = TOS (mmol $H_2O_2$/l)/TAS (mmol Trolox/l) [17].

### 2.7. Measurement of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level

The erythrocyte sedimentation rate (ESR) was measured through the Westergren method (mm/h) and the serum C-reactive protein (CRP) level was determined with the help of nephelometry (mg/dl). The RF titers were also measured through the nephelometric immunoassay method (IU/ml).

## 3. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 13.0 software for Windows (SPSS Inc., Chicago, IL, USA). All quantitative variables were estimated using measures of central location (i.e. mean and median) and measures of dispersion (i.e. standard deviation (SD)). Data normality was checked using the Kolmogorov–Smirnov tests of normality.

One-way analysis of variance (ANOVA) was used in comparing the values in between the groups. Tukey's HSD post hoc test was used in determining the differences in between groups.

Pearson's correlation test was used for evaluating correlations between data. In all comparisons and correlations, $p < 0.05$ was accepted as significant.

## 4. Results

### 4.1. The demographics and laboratory data of the groups

There were no significant differences between the mean ages (Group 1: 4.1 ± 1.9, Group 2: 3.9 ± 1.8, Group 3: 3.6 ± 1.6), and genders (Group 1 (11F, 19M), Group 2 (8F, 14M), Group 3 (7F, 13M)) of the two groups ($p > 0.05$) (Table 2). There were no significant differences between the mean weights and heights of the three groups ($p > 0.05$) (Table 2). There were no significant differences between the basal metabolic index (BMI), BMI SDS, and BMI% parameters of the three groups ($p > 0.05$) (Table 2). There were no significant differences between the hemoglobin (Hb), hematocrit (Htc), and mean corpuscular volume values of the three groups ($p > 0.05$) (Table 2).

There were no significant differences between the C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values of Group 1 (PFAPA) and Group 2 (NTP) ($p > 0.05$) (Table 2).

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values of Group 1 (PFAPA) were significantly higher than those of Group 3 (CNT) ($p < 0.01$ and $p = 0.003$, respectively) (Table 2).

**Table 2**
Characteristics of the study groups.x.

| Characteristic | Group 1 ($n=31$) (PFAPA) | Group 2 ($n=22$) (NTP) | Group 3 ($n=20$) (CNT) | One-way ANOVA |
|---|---|---|---|---|
| Sex (male/female) | 11/19 | 8/14 | 7/13 | $p=0.523^{\psi}$ |
| Age (years) | 4.1 ± 1.9 | 3.9 ± 1.8 | 3.6 ± 1.6 | $p=0.317^{\psi}$ |
| Height (m) | 103.6 ± 15.8 | 105.8 ± 16.37 | 99.2 ± 12.7 | $p=0.316^{\psi}$ |
| Weight (kg) | 17.4 ± 5.2 | 16.6 ± 4.9 | 15.7 ± 4.2 | $p=0.239^{\psi}$ |
| Basal metabolic index | 16.0 ± 1.9 | 16.3 ± 2.0 | 15.8 ± 1.7 | $p=0.682^{\psi}$ |
| Basal metabolic index SDS | 0.09 ± 1.1 | 0.07 ± 0.9 | 0.12 ± 1.2 | $p=0.583^{\psi}$ |
| Basal metabolic index (%) | 48.53 ± 5.7 | 48.42 ± 4.9 | 48.7 ± 3.6 | $p=0.978^{\psi}$ |
| Hemoglobin (g/dl) | 11.5 ± 2.2 | 11.4 ± 1.8 | 11.7 ± 1.2 | $p=0.544^{\psi}$ |
| Hematocrit (%) | 34.8 ± 2.2 | 34.2 ± 3.1 | 35.6 ± 2.8 | $p=0.334^{\psi}$ |
| Mean corpuscular volume ($\mu^3$) | 75.6 ± 3.0 | 76.0 ± 4.6 | 74.1 ± 5.5 | $p=0.245^{\psi}$ |
| C-reactive protein (mg/l) | 2.4 ± 3.5 | 2.1 ± 2.8 | 0.3 ± 0.3 | $p=0.007^{¥}$ |
| Erythrocyte sedimentation rate (mm/h) | 21.6 ± 11.1 | 22.9 ± 7.3 | 13.3 ± 5.6 | $p=0.003^{¥}$ |

x For the comparison between groups, the one-way analysis of variance (ANOVA) test was used.
¥ Significance level obtained ($p < 0.05$).
ψ Insignificance level obtained ($p > 0.05$).

**Table 3**
Comparison of DNA damage and oxidative status parameters between groups.x.

| Parametres | Groups | Mean ± Std Dev | One-way ANOVA | Between group | Tukey's HSD (post hoc test) |
|---|---|---|---|---|---|
| Mononuclear leukocyte DNA damage (arbitrary units) | Group 1 ($n=31$) | 26.70 ± 9.62 | $p=0.001$ | Grp 1 vs Grp 2 | $p=0.007$ |
| | Group 2 ($n=22$) | 18.51 ± 8.04 | | Grp 1 vs Grp 3 | $p=0.005$ |
| | Group 3 ($n=20$) | 17.10 ± 7.35 | | Grp 2 vs Grp 3 | $p=0.309$ |
| Total antioxidant status (TAS) (mmol Trolox equiv./l) | Group 1 ($n=31$) | 0.07 ± 0.03 | $p=0.001$ | Grp 1 vs Grp 2 | $p=0.013$ |
| | Group 2 ($n=22$) | 0.20 ± 0.19 | | Grp 1 vs Grp 3 | $p=0.008$ |
| | Group 3 ($n=20$) | 0.16 ± 0.11 | | Grp 2 vs Grp 3 | $p=0.527$ |
| Total oxidant status (TOS) ($\mu$mol $H_2O_2$ equiv./l) | Group 1 ($n=31$) | 14.59 ± 3.90 | $p=0.005$ | Grp 1 vs Grp 2 | $p=0.001$ |
| | Group 2 ($n=22$) | 5.47 ± 1.82 | | Grp 1 vs Grp 3 | $p=0.001$ |
| | Group 3 ($n=20$) | 4.16 ± 2.25 | | Grp 2 vs Grp 3 | $p=0.291$ |
| Oxidative stress index (OSI) (arbitrary unit) | Group 1 ($n=31$) | 2.08 ± 0.37 | $p=0.001$ | Grp 1 vs Grp 2 | $p=0.001$ |
| | Group 2 ($n=22$) | 0.27 ± 0.04 | | Grp 1 vs Grp 3 | $p=0.001$ |
| | Group 3 ($n=20$) | 0.26 ± 0.03 | | Grp 2 vs Grp 3 | $p=0.473$ |

x For the comparison between groups, the one-way analysis of variance (ANOVA) test was used ($p<0.05$ was accepted as statistically significant).
Tukey's HSD was administered as a *post hoc* test to identify within-group differences ($p<0.016$ was accepted as statistically significant).

### 4.2. DNA damage and oxidative stress

DNA damage was significantly higher in Group 1 (PFAPA) than in Group 2 (NTP) and Group 3 (CNT) ($p=0.007$ and $p=0.005$ respectively) (Table 3). There were no significant differences between the DNA damage values of Group 2 (NTP) and Group 3 (CNT) ($p=0.309$) (Table 3).

TAS values were significantly lower in Group 1 (PFAPA) than in Group 2 (NTP) and Group 3 (CNT) ($p=0.013$ and $p=0.008$ respectively) (Table 3). There were no significant differences between the TAS values of Group 2 (NTP) and Group 3 (CNT) ($p=0.527$) (Table 3).

TOS values were significantly higher in Group 1 (PFAPA) than in Group 2 (NTP) and Group 3 (CNT) ($p=0.001$ and $p=0.001$, respectively) (Table 3). There were no significant differences between the TOS values of Group 2 (NTP) and Group 3 (CNT) ($p=0.291$) (Table 3).

OSI values were significantly higher in Group 1 (PFAPA) than in Group 2 (NTP) and Group 3 (CNT) ($p=0.001$ and $p=0.001$, respectively) (Table 3). There were no significant differences between the OSI values of Group 2 (NTP) and Group 3 (CNT) ($p=0.473$) (Table 3).

### 4.3. Correlation analysis

There was a significant negative correlation between DNA damage and the TAS level in Group 1 (PFAPA) ($r=.392$, $p=0.029$).

There was a significant positive correlation between DNA damage and the OSI level in Group 1 (PFAPA) ($r=.397$, $p=0.027$).

There was a significant positive correlation between DNA damage and the TOS level in Group 1 (PFAPA) ($r=.739$, $p=0.016$).

No significant correlations were observed between DNA damage and the TAS, TOS, and OSI values in Group 2 (NTP) ($p=0.294$, $p=0.409$, and $p=0.358$, respectively).

No significant correlations were observed between DNA damage and the TAS, TOS, and OSI values in Group 3 (CNT) ($p=0.179$, $p=0.780$, and $p=0.528$, respectively).

There were significant positive correlations between DNA damage, and CRP and ESR values in Group 1 (PFAPA) ($r=.317$, $p=0.045$ and $r=.298$, $p=0.042$, respectively).

## 5. Discussion

PFAPA syndrome is a systemic disease of unknown etiopathology which presents with regularly occurring periodic high fever, and which has not yet been associated with any geographic or ethnic factors. Although research on the prevalence of PFAPA syndrome is lacking, it is known that the syndrome is quite widespread in the population. It is seen mostly in children below 5, and the gender preference favors males [18]. In our study the males (#19) outnumbered females (#11) in the PFAPA group, and that finding was compatible with relevant literature. The syndrome proceeds with recurring attacks, and the attack frequency decreases with age [6]. There are also studies which report PFAPA cases in the adult age group [19].

Recurring fever episodes are the primary cause of morbidity in PFAPA patients. Those patients have normal growth and development curves for their age groups, and exhibit no long-term sequelae in later years.

The pathophysiology of the disease, investigated by numerous studies, has not been elucidated yet. The most probable mechanism seems to be autoimmunity [20]. All studies focused on deciphering the genetic basis of the disease were inconclusive; however, a recent study proposed a probable familial transmission [21]. Case reports in medical literature also indicated a positive family history [22,23]. One study demonstrated heterozygous mutations in the MEFV gene [24]. In our study, the PFAPA group had a significantly higher rate of DNA damage as compared to the control group ($p<0.001$) (Table 2). That is a novel finding which indicates the role of DNA pathology in the pathophysiological makeup of the disease, and supports relevant medical literature.

Acute phase reactants are elevated in PFAPA, especially during the attack periods. Specifically, the CRP and ESR are increased at febrile episodes [4]. In our study, CRP and ESR values of the PFAPA group were significantly higher than those of the control group (Table 2). Various studies reported increases in cytokine concentrations (IFN-$\gamma$ and TNF-$\alpha$) during attacks [25]. Also, histopathological assessment indicated chronic inflammation of the tonsils [26]. Based upon the above findings, we evaluated the oxidative status, for elucidating the pathophysiological characteristics of PFAPA. The TOS and OSI values of Group 1 (PFAPA) were significantly higher than those of Group 2 (NTP) and Group 3 (CNT) (Table 2). The TAS values of the PFAPA group were significantly lower than those of the Group 2 (NTP) and Group 3 (CNT) (Table 2). It was the first time in medical literature that the oxidative balance in PFAPA patients was assessed, and the increase of oxidative stress was demonstrated in PFAPA. Our results indicate that there is an ongoing oxidative damage in PFAPA patients, and the responding antioxidant mechanisms are insufficient in overcoming that damage.

Recent studies in medical literature revealed a significant correlation between oxidative stress and DNA damage [9,10]. In

our study, significant positive correlations between DNA damage and the TOS and OSI parameters, and significant negative correlations between DNA damage and TAS values were detected in the PFAPA group ($p < 0.05$). Those findings indicated that oxidative stress, generated by chronic inflammation, induced the DNA damage. According to the results of our study, we think that DNA damage induced by oxidative stress, brought about by inflammation, appears to be involved in the pathophysiology of the PFAPA syndrome.

Increased oxidative stress has been shown not only in PFAPA specifically but also in many autoimmune and chronic diseases [27,28]. Therefore, we do not think there is enough evidence to say that the increased oxidative stress is the cause or the result of PFAPA. However, in PFAPA syndrome the total oxidant status is increased, antioxidant parameters are decreased and consequently DNA damage has to be seen. This situation suggests to us that PFAPA attacks may be reduced or treated if oxidative stress can be prevented.

There are some studies in order to elucidate the pathogenesis of PFAPA and diagnostic biomarkers to defining the attacks. Yamazaki et al. showed that CD64 is a reliable diagnostic biomarker to define the disease during attacks [29]. Stojanov et al. noted that inhibition of IL-1 during attack can be effective in the treatment and IP-10/CXCL10 is also used as a biomarker [30]. Also Førsvoll et al. showed that CXCL10 as a useful biomarker for diagnosing PFAPA [31]. In our study DNA damage and oxidative stress values are increased meaningfully. We think that additional clinical trials to use oxidative stress and DNA damage parameters as a biomarker are needed.

The limitation of our study was the absence of an evaluation related to the biological parameters of autoimmunity, which has been the most emphasized pathophysiological cause of PFAPA syndrome.

The strengths of our study can be listed as:

- The biochemical methods we have employed in evaluating DNA damage and oxidative stress were acknowledged, efficient and reliable methods.
- The correlation analyses between inflammation, DNA damage and oxidative stress have yielded meaningful results.

Further studies on this subject may focus on investigating DNA damage and oxidative status during afebrile periods, or in cases who are free of febrile attacks, like cases whose attacks terminated after tonsillectomies or cases in remission.

## 6. Conclusion

Our study demonstrated that in patients with PFAPA syndrome, oxidative stress and DNA damage were increased. This finding is a first in medical literature, and implies that DNA damage might have been induced by the increased oxidative stress, triggered by chronic inflammation. Termination of the inflammation is of prime importance for preventing the severe consequences of DNA damage brought forward by chronic inflammation.

## Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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