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Cytogenetic biomonitoring in children with chronic tonsillitis: Micronucleus frequency in exfoliated buccal epithelium cells

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KEYWORDS
Tonsillitis;
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Summary

Objective: To investigate the possible harmful cytogenetic effects associated with chronic tonsillitis by analyzing the micronucleus frequency and other nuclear abnormalities in exfoliated buccal epithelial cells.
Materials and methods: The study consisted of 20 children with chronic tonsillitis, and 20 control subjects with similar age and sex. The ages ranged between 5 and 12 years old (mean age: 7.5). The patients were diagnosed as having chronic tonsillitis on the basis of history, throat culture and clinical examinations. Buccal cell samples were collected with a wooden spatula. The samples were then applied to clean microscope slides. Smears were air dried and fixed in methanol:acetic acid. Then slides were stained by the Feulgen reaction technique. Three slides were prepared for each subject and 1000 cells were evaluated per slide to determine the frequencies of micronucleus and other nuclear abnormalities (binucleats, karyorrhexis and karyolysis). Statistically, Mann—Whitney *U*-test was used to analyze and compare the data.
Results: The mean micronucleus frequencies in patient and control groups were 5.29 ± 1.67 and 1.58 ± 0.33, respectively. In the patient group, mean binucleus, karyorrhexis and karyolysis frequencies were 3.13 ± 1.2, 2.04 ± 0.64, and 1.74 ± 0.47, respectively. However, in the control group, mean binucleus, karyorrhexis and karyolysis frequencies were 1.43 ± 0.47, 1.26 ± 0.45, and 0.88 ± 0.27, respectively. The mean frequencies of all parameters in the patient group were higher than the control values, and the difference was found to be statistically significant ($p < 0.001$).

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Conclusion: Our results revealed that children with chronic tonsillitis could be under risk of significant cytogenetic damage.

1. Introduction

Diagnostic cytopathology is a useful and well-established clinical tool to determine the impact of environmental, genetic, medical treatment and life-style factors on genomic stability. Micronucleus (MN) test is one of the modern techniques to monitor these hazardous effects on genetic material. This test provides an indirect and sensitive measure of chromosomal breakage or missegregation, and has received increased attention as a sensitive biologic marker of genotoxic exposure [1]. Micronuclei originate from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division [2]. They are small, round to oval bodies found within the cytoplasm but outside the main nucleus [3]. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations during mitosis [2,4].

The palatine tonsils are the first line of defense against the invasion of viruses, microbes and irritants into the airways [5]. Recurrent tonsillitis and reactive hypertrophy can become serious clinical problems. Although numerous anatomical, pathological, biological, microbiological, and immunological studies have been conducted on chronic/recurrent tonsillitis, the etiopathogenetic mechanisms underlying these entities are still unknown. Also, recurrent and chronic tonsillitis may cause many systemic negative effects on the body including rheumatic fever, glomerulonephritis, endocarditis, growth retardation, etc. Additionally, numerous medications such as antibiotics, analgesic and anti-inflammatory drugs are prescribed for recurrent or chronic tonsillitis, but there is no accepted consensus on the use of these drugs (duration, dosage, combination therapy, etc.). We hypothesized that these potential harmful systemic factors may also lead to a detoriation in the cellular nuclear component. The aim of the present study was to investigate the possible genotoxic effects associated with chronic ton-sillitis by analyzing MN frequencies and other nuclear abnormalities in exfoliated buccal epithelial cells.

2. Materials and methods

2.1. Subjects

The study consisted of 20 children with chronic tonsillitis, and 20 control subjects with similar age and sex. The ages ranged between 5 and 12 years old (mean age: 7.5). Male female ratio was 3:2. All the patients and/or their parents had complaints of sore throat and fever attacks at least one year. The patients were diagnosed as having chronic tonsillitis on the basis of history, throat culture (group A β-hemolytic streptococcus infection at least five times per year), and clinical examinations. They were healthy otherwise. All the patients underwent a tonsillectomy operation under general anesthesia with cold dissection methods. Control subjects were selected among the children, who admitted to our outpatient clinic with no adenotonsillar disease, upper and lower respiratory tract infection and obstruction. Exclusion criteria included head and neck malformation, patients with identified syndromes and neurological or any other systemic diseases, recent infection, and medication. All subjects and their parents gave written informed consent.

Prior to the study, all the participants filled in a detailed questionnaire including the standard demographic questions such as diet, parents' smoking habit, medical and family history and occupational status.

2.2. Cytogenetic analysis

Buccal cell samples were collected before the operation with a wooden spatula. The samples were then applied to clean microscope slides. Smears were air dried and fixed in methanol:acetic acid (3:1). Then slides were stained by the Feulgen reaction technique according to Stich and Rosin

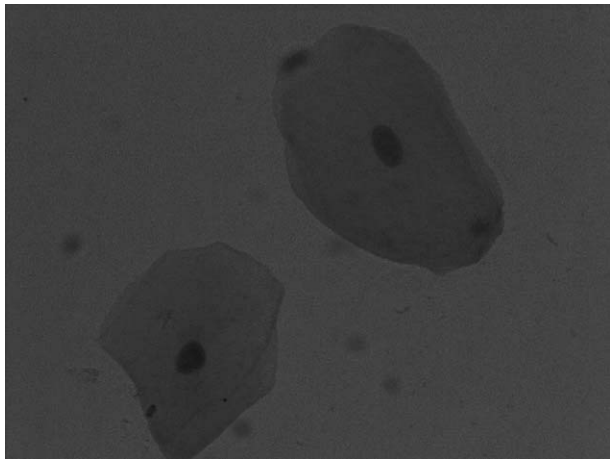

Fig. 1 Buccal epithelial cells containing normal nuclei.

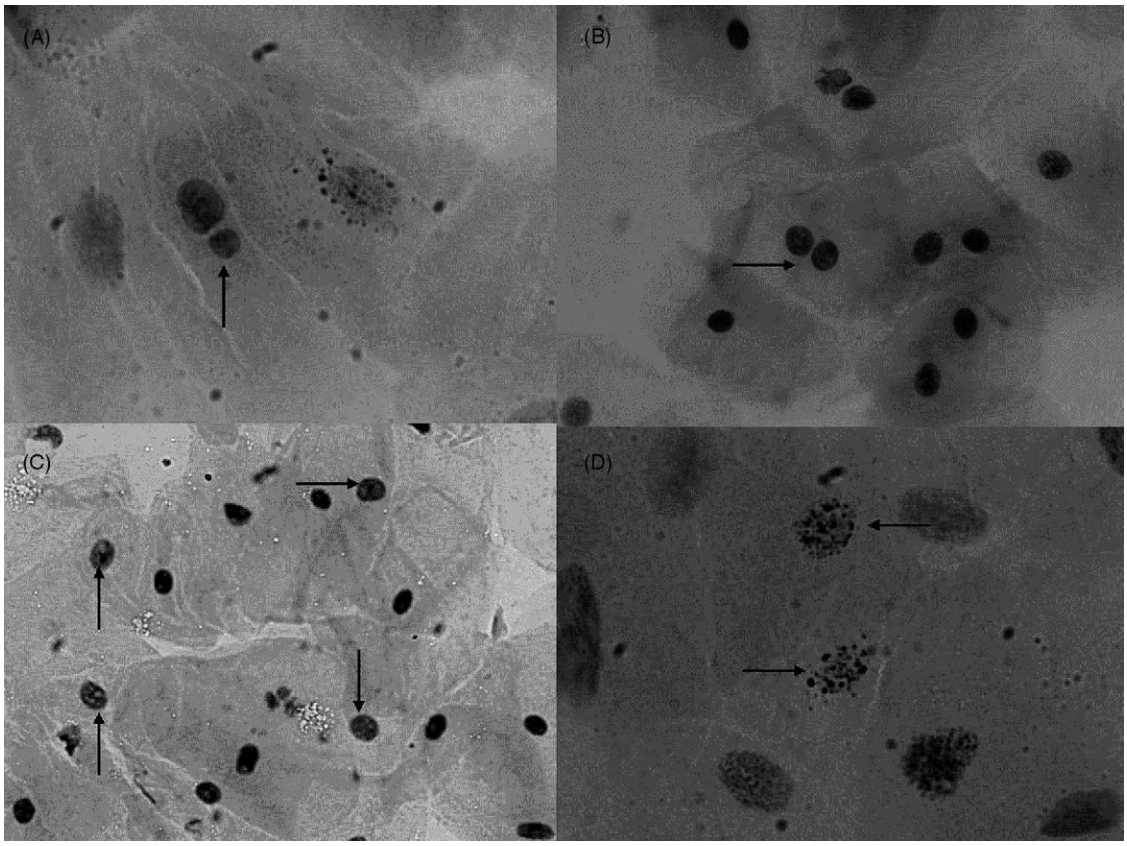

Fig. 2 (A) Arrow indicates the micronucleus formation close to the main nucleus; (B) arrow shows an epithelial cell containing binucleus; (C) arrows indicate nuclear karyorrhexis; (D) arrows indicate nuclear karyolysis.

[6]. Three slides were prepared for each subject and 1000 cells were evaluated per slide to determine the MN frequencies by one of the authors (A.C.) [4]. Fig. 1 shows the normal nuclear formation of a buccal epithelial cell.

The following criteria for MN analyses were used in buccal epithelial cells: A MN must be (i) less than one-third of the diameter of the main nucleus; (ii) on the same plane of focus; (iii) have the same color, texture and refraction as the main nucleus; (iv) have a smooth, oval or round shape; (v) be clearly separated from the main nucleus (Fig. 2A) [4]. Nuclear abnormalities were classified according to Tolbert et al. [7]. Cells with two nuclei were considered as binucleats (BN) Fig. 2B). Nuclei fragmented into irregular pieces were scored as karyorrhexis (KR) (Fig. 2C). Nuclear dissolution, in which a Feulgen-negative, ghost-like image of the nucleus remains was evaluated as karyolysis (KL) (Fig. 2D).

2.3. Statistical analysis

All the data were expressed as the mean ± standard error of the mean. Mann—Whitney *U*-test was used to analyze and compare the data. The level of significance was set at 5% ($p < 0.05$).

3. Results

The mean MN frequencies in patient and control groups were 5.29 ± 1.67, and 1.58 ± 0.33, respectively (Fig. 3). In the patient group, mean BN, KR and KL frequencies were 3.13 ± 1.2, 2.04 ± 0.64, and 1.74 ± 0.47, respectively (Figs. 4—6). However, in the control group, mean BN, KR and KL frequencies were 1.43 ± 0.47, 1.26 ± 0.45, and 0.88 ± 0.27, respectively. The mean frequencies of all parameters in the patient group were higher than the

Table 1 The frequencies (‰) of micronuclei and other nuclear abnormalities in the patient and control groups

Group	*n*	MN	BN	KR	KL	*p*-value
Patient	20	5.29 ± 1.67	3.13 ± 1.2	2.04 ± 0.64	1.74 ± 0.47	0.000
Control	20	1.58 ± 0.33	1.43 ± 0.47	1.26 ± 0.45	0.88 ± 0.27	0.000

MN: micronuclei, BN: binucleats, KR: karyorrhexis, KL: karyolysis.

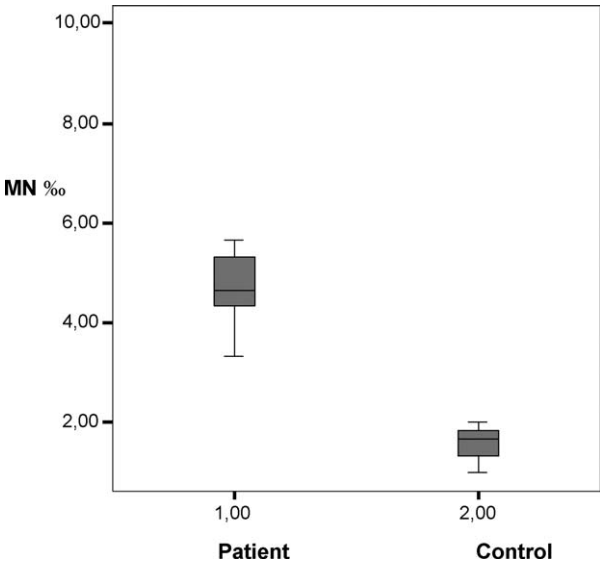

Fig. 3 Box-plot diagram shows the distribution of MN frequencies (%) in the patient and control groups.

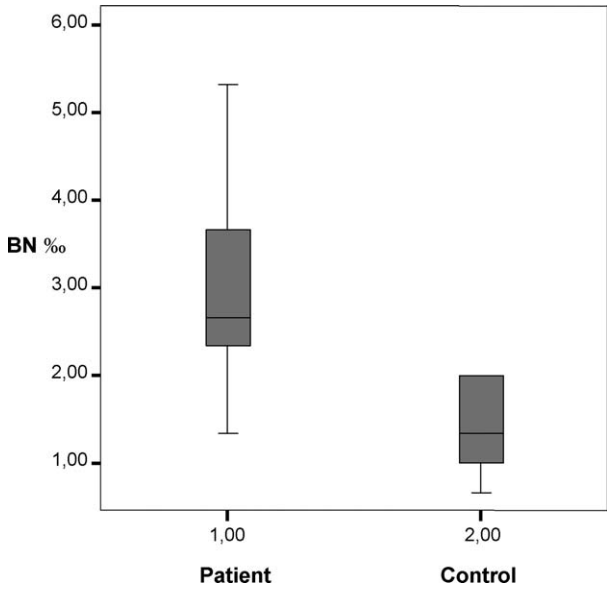

Fig. 4 Box-plot diagram shows the distribution of BN frequencies (%) in the patient and control groups.

control values and the difference was found to be statistically significant ($p < 0.001$). Table 1 summarizes the statistical results. Also, there was no marked difference between the groups according to the questionnaire data.

4. Discussion

This study clearly demonstrated that MN frequencies and other nuclear abnormalities were significantly increased in children with chronic tonsillitis.

Micronuclei have been proposed as a useful biomarker to assess cytogenetic damage in biomonitoring studies, both using peripheral lymphocytes and epithelial cells. Studies about MN have been carried out in populations exposed to ionizing radiation, smokers, gas station workers, workers exposed to pesticides, styrene, ethylene dioxide, polycyclic aromatic hydrocarbons, and patients with different kinds of epithelial cancers (such as oral, esophageal, lung, bladder, etc.) [4,8—11]. According to our knowledge and extensive Medline search, we could not see any study, which investigates the MN frequency in children with chronic tonsillitis.

The buccal epithelium is composed of four strata including the basal cell layer, prickle cell layer, intermediate and superficial layers. This epithelium maintains itself by a system of continuous cell renewal in which new cells produced by mitosis in the basal layer migrate to the surface to replace those that are shed. Thus, the mucosa is composed of progenitor and maturing cell populations [4,12]. The average reported healthy population MN frequency in exfoliated epithelial cells is 1—3 per 1000 cells [2]. In our study, we found MN frequency in the patient group 5.29 ± 1.67 per 1000 cells and 1.58 ± 0.33 per 1000 cells in the control group. The main advantage of the MN assay is to be a simple, practical, inexpensive and non-invasive screening technique. Although variability of MN assessment may arise due to cell kinetics, sampling and methodological differences (such as counted cell numbers), but many studies have shown that MN test in exfoliated epithelial cells is an effective method to detect unstable chromosomal aberrations [7,11,13,14]. Also, in contrast to the MN frequency in lymphocytes, there were no consisted sex or age effects on the MN frequency in exfoliated cells [2]. However, micronucleus test may be affected by dietary factors such as folate and Vitamin B12 deficiencies [2].

Children may express increased susceptibility to environmental hazards, chronic infection and inflammation, dietary factors, and long-term medication due to differences in the uptake, metabolism, distribution and excretion of mutagens [15]. Micronucleus studies in children with non-neoplastic (two studies) and neoplastic diseases (four studies) revealed an increased MN frequency compared to healthy controls [15]. Urazova et al. found a threefold increase in MN frequency in children with acute and convalescent mononucleosis [16]. The other study was performed in children with Fanconi anemia and infants with Down syndrome [17]. Interestingly, Epstein—Barr virus (EBV) is one of the viruses mostly involved in recurrent bouts of acute tonsillitis [18]. The epithelial cells of the oropharynx are

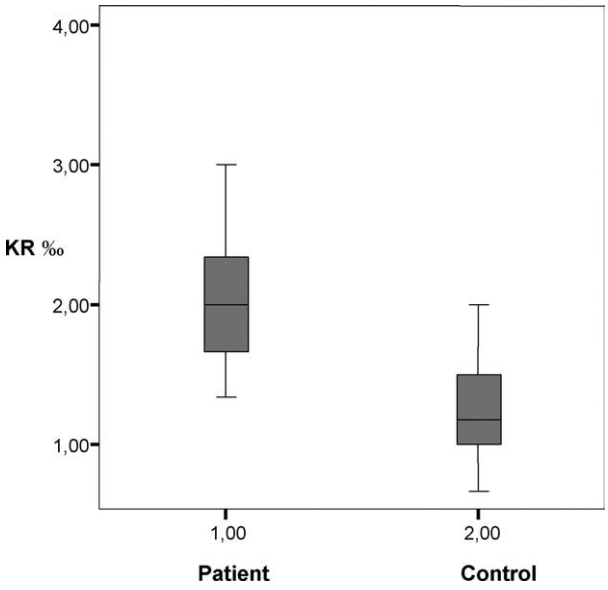

Fig. 5 Box-plot diagram shows the distribution of KR frequencies (%) in the patient and control groups.

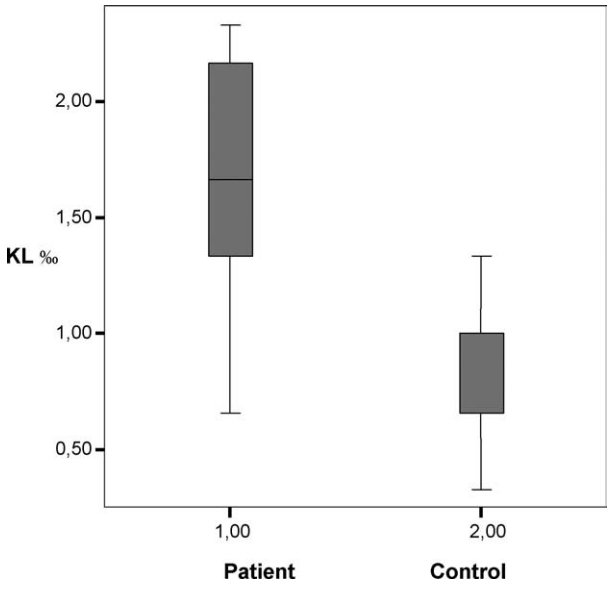

Fig. 6 Box-plot diagram shows the distribution of KL frequencies (%) in the patient and control groups.

the first targets of infection by EBV. Endo et al. demonstrated that EBV can persist in the tonsils in a latent state ready to provoke acute infections and could act through its lymphoproliferative ability to provoke hypertrophy [18].

Analysis of buccal exfoliated cells also provides evidence of other nuclear abnormalities such as binucleats (presence of two nuclei within a cell), karyorrhexis (nuclear fragmentation) and karyolysis (nuclear dissolution) [7]. Binucleus formation is considered as an indicator of cytotoxicity, while karyorrhexis and karyolysis are considered as an indicator of apoptosis [4,7]. In our study, we also demonstrated a significant difference in nuclear abnormalities between the patient group and controls.

Our previous studies about inflammation and chronic tonsillitis revealed that inflammatory cytokines, reactive oxygen species and L-arginine:Nitric oxide pathway are involved in the development of chronic adeno-tonsillar disease [19—21]. The excess amount of these substances may show cytotoxic and genotoxic effects on the oropharyngeal epithelium, and all of them have a role on the development of chronic tissue inflammation and carcinogenesis [19—22]. Also, long-term medication and nutritional factors in children with chronic tonsillitis may have a negative effect on the integrity of the epithelium [23]. In conclusion, our results revealed that children with chronic tonsillitis could be under risk of significant cytogenetic damage. We suggested that increased nuclear damage may be due to EBV and/or other microorganisms, systemic inflammation, long-term medication and dietary factors. It should also be kept in mind that there has been no published study demonstrating the increased risk of malignancy in children with chronic tonsillitis yet. Thus, further in vivo and in vitro studies need for showing the importance of MN test and nuclear abnormalities and their causative mechanisms in the chronic tonsillitis.

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