Correlation of Buccal Micronucleus with Disease Activity Score Using Buccal Micronucleus Cytome Analysis (BMCA) in Systemic Lupus Erythematosus

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Abstract

Background: A disease activity score obtained by using systemic lupus erythematosus disease activity index (SLEDAI) has traditionally been a reliable method to assess the SLE status of patients. More recently, a buccal micronucleus cytome assay (BMCA) has been developed for use as a biomarker of DNA damage in patients with SLE. There has been a very limited number of studies pertaining to the oral lesions in Arab population suffering from SLE in Asir region. Hence, it became utmost important to study epidemiological data of oral mucosal lesions in SLE patients. Distribution of oral lesions in SLE patients could also be helpful in better management of oral complications. Aim: The aim of this study was to determine the existence of a correlation between SLEDAI score and the degree of micronuclei (MN) formation using BMCA. Materials and Methods: After thorough oral examination of adult Saudi SLE patients of Asir hospital and that of healthy control subjects, the subjects underwent BMCA from normal unaffected bilateral buccal mucosae. Results: Pearson’s correlation test showed that MN count did not significantly correlate with either disease activity or duration of SLE. Conclusions: Controlled state SLE does not lead to a significant increase in MN formation. Thus, the occurrence of premalignant lesions in the oral cavity could be minimized using proper management protocols.

Key Words: Buccal epithelial cells, disease activity score, micronucleus, systemic lupus erythematosus

Introduction

Complex clinical and molecular manifestations make systemic lupus erythematosus (SLE) an archetypal autoimmune disease that affects a large number of individuals. Proper management and assessment of the disease is essential to prevent organ damage and improve quality of life. Here, we describe the constituents of the broad assessment of SLE, including monitoring disease activity, and its correlation with micronuclei (MN) formation. The most appropriate laboratory tests and indices in current clinical practice are reviewed.[1]

Previous studies have shown the prevalence of SLE in Saudi population to be less as compared with other Asian countries. Prevalence rates usually fall within 30–50 per 100,000 people.[2] Three Asian countries, India, Japan, and Saudi Arabia, showed the lowest prevalence, ranging from 3.2–19.3 per 100,000 population.

Several important mechanisms, such as apoptosis, are involved in the pathogenesis of SLE. A previous study demonstrated an increase in lymphocyte apoptosis and impaired removal of apoptotic cells in SLE. In addition, apoptosis was found to be greater in active SLE as compared with inactive SLE.[3]

Because no specific measure can describe status in all SLE patients, standardized indices for measuring SLE disease activity have been formulated. The most widely used, and simplest to use, tools for measuring the disease activity score in SLE are the Physicians’ Global Assessment and the SLE disease activity index (SLEDAI).[4,5] Twenty-four features attributed to lupus having individual weighted scores are listed in the SLEDAI. More weight is given to severe manifestations

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involving renal, neurologic, and vascular systems rather than cutaneous manifestations, with a maximum possible score of 105. More recent revisions have been proposed, which place more emphasis on the ongoing disease rather than only new or recurrent activity (known as SELENA-SLEDAI and SLEDAI 2001). [6]

The buccal micronucleus cytome assay provides baseline information to identify patients at high risk of progressing to malignancy by providing information about nuclear injury during the earliest microinvasive phase. [7] Micronuclei can provide information about quantitative damage at the genomic level in vivo for cells such as erythrocytes, lymphocytes, and exfoliated epithelial cells. The main target cells of MN are rapidly dividing exfoliated cells. The MN assay is widely preferred over a cytogenetic assay as an initial screening as it is simple, noninvasive, and less time consuming, and does not require ex vivo nuclear division. [8] The assay involves staining exfoliated buccal epithelial cells and observing the smear under a light microscope. The ease with which samples can be obtained makes BMCA a good choice for screening groups of individuals that are undergoing treatment for SLE. A database search did not identify any studies that addressed the effect of SLE on buccal mucosal cells. It is reported that MN frequency has increased in patients with cancer, [9] neurodegenerative, [10] cardiovascular disease, and diabetes. [11] Moreover, increased MN frequency in normal subjects reflect genomic instability and a predictive role for future occurrence of cancer. In order to have validated data to help predict future occurrence of oral lesions in SLE patients, it was mandatory for the authors to access clinically normal appearing mucosa.

Therefore, in the current study, we aimed to evaluate the effect of SLE on normal buccal mucosal cells using BMCA and then correlate these findings with disease activity score.

**Materials and Methods**

**Patient selection**

We performed a cross-sectional study involving 35 adult Saudi patients who were diagnosed with SLE at least 1 year prior to examination and were being managed at Asir Central Hospital. All the patients with SLE were assigned to the research group (RG; n = 35) and were diagnosed with SLE according to American College of Rheumatology criteria, and managed at Rheumatology clinics within Asir Central Hospital. A skilled rheumatologist assigned an appropriate SLEDAI score after retrieving the patients’ medical records. Patients underwent thorough oral mucosal and dental examinations under adequate lighting and magnification. Patients were in the age range of 18–50 years.

Control subjects were assigned to the control group (CG; n = 36). These individuals were not using any form of mouth rinse and had discontinued the use of any orthodontic appliances at least 1 year before the start of the study. Smokers and individuals with oromucosal lesions or who were using topical agents were excluded from the study. The age range of the control population was 21–50 years. The study was registered with Academic Affairs for Training and Research, Asir Central Hospital and ethical approval was obtained from the Internal Review Board Committee of Asir Central Hospital. (REC# 2018-03-12 (May 22, 2018))

Buccal mucosal scrapings were obtained after due informed consent from the patients. The site selected was bilateral normal appearing buccal mucosa.

**Buccal micronucleus cytome assay**

BMCA is a minimally invasive, chairside investigation performed by scraping buccal epithelial cells, with the goal of identifying dysplastic changes. Patients were asked to rinse their mouth with water prior to sample collection. Buccal mucosal scrapings were obtained from normal unaffected buccal mucosa using a wooden spatula. Samples were then smeared directly onto a glass microscope slide and fixed with absolute ethanol. Two slides were prepared for each subject, and were stained with DNA-specific Feulgen-theonin. MN frequencies were determined from a total of 1,000 cells. Scoring criteria for the various distinct cell types and nuclear anomalies were evaluated in the BMCA, as described by Tolbert et al. [12]

An oral pathologist examined the samples under blinded conditions under light microscope at 100× magnification. Overlapping stained epithelial cells and clusters were excluded from the analysis. An average of 2,000 epithelial cells was observed for each individual. Scoring was done according to criteria established by Thomas et al. [13]

The morphological features of MN cells as described by Thomas et al. are: (a) cells with both a main nucleus and MN, (b) MN being round or oval with similar staining intensity as the main nucleus, (c) MN usually 1/3 to 1/16 diameter of the main nucleus, (d) MN found within the cellular cytoplasm, and (e) counting performed only in basal and differentiated cells.

**Statistical analysis**

BMCA data obtained were correlated with clinical data from the sample population.

On completion of data recording, the distribution was calculated and results were statistically analyzed using SPSS software (SPSS Inc., Chicago, IL, USA), where \( P < 0.05 \) was considered significant.

**Results**

A descriptive analysis was carried out using the obtained data. Table 1 shows sample distribution according to
gender in RG and CG. The mean frequency (or number) of MNs in a total of 2,000 cells in patients with SLE (RG) was 5.25, whereas in healthy individuals (CG), this number was 4.77. The mean value of MN cells observed in RG was compared with that for CG. Table 2 shows the mean, median, standard deviation, and standard error of the analysis at a 95% confidence interval. Nonparametric test results showed that the difference in the mean values of MN in both groups was not statistically significant ($P = 0.057$). Pearson’s correlation test showed that there was no significant correlation between MN count and disease activity ($P = 0.079$) or duration of SLE ($P = 0.934$).

**Discussion**

**Rationale for undertaking this research**

Because of the diversity of SLE expression, prevalence studies report varied outcomes for different ethnic groups. Data from Taif region of Saudi Arabia region report a prevalence of 19.28 per 100,000 individuals. The most common oral manifestations of SLE are oral ulcers, which account for 39.1% of all cases. Furthermore, this has a significant impact on the patients’ quality of intraoral health compared with healthy individuals ($P = 0.011$). Up to 5.0% of oral lesions in SLE have been shown to undergo malignant transformation. Because of the increasing prevalence of SLE in Arab populations, which can be attributed to globalization, it is imperative to devise a simple and clinically applicable chairside screening tool that will provide information about genotoxicity and cytotoxic effects on the oral mucosa.

Initial studies on micronuclei during 1980s were targeted towards genotoxic effects using exfoliated buccal mucosa cells. Later, the micronuclei from buccal cells were utilized to study effects of a number of chemotherapeutic agents in cancerous and precancerous lesions.

The oral epithelium keeps up itself by constant cell regeneration, whereby new cells created in the basal layer by mitosis relocate to the surface supplanting those that are shed. This layer contains stem cells that may express genetic damage as MN formation amid nuclear division. Some cells may deteriorate containing condensed chromatin, fragmented nuclei (karyorrhectic cells), pyknotic nuclei, completely lose their nuclear material (karyolytic), may be blocked in a binucleated stage or exhibit nuclear buds. These biomarkers can be observed in lymphocyte and buccal cell systems and thus provide a more comprehensive assessment of genome damage and cell death.

Employing BMCA in cytotoxicity studies may improve the predictive value of the assay, and we believe that this deserves further investigation in the context of SLE. Al-rawi et al. found that a BMCA score greater than 4 had the highest accuracy, sensitivity, and specificity, with an almost 100% positive predictive value for the diagnosis of SLE.

The results of our current study showed no significant difference in the mean values of MN frequency in patients with SLE (RG) versus healthy individuals (CG) ($P = 0.057$). The variation in our results could be attributed to the fact that patients were undergoing medical treatment before the start of the study, and therefore their SLEDAI scores were under control. Our study utilized SLEDAI scores for the categorization of patients because of its practicality and simplicity of use, making it easy to monitor disease status and treatment outcomes.

We did not find any studies that described the histopathological picture from a normal-appearing oral mucous membrane in patients with SLE. Only one study utilized MN formation as criteria to assess oral mucosal damage. Similarly, our present study utilized MN acquired from normal oral mucosa. We showed that MN values did not significantly correlate with the duration of SLE or disease activity. This is in contrast to reports that found a significant increase in MN counts in the diseased state. However, such studies did not consider disease activity scores based on SLEDAI.

In the present study, the frequency of MN in patients with SLE (RG) compared with healthy individuals (CG)

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**Table 1: Distribution of individuals in Control and Risk Group**

| Risk Group | Male | Female | Total |
|------------|------|--------|-------|
| RG         | 9    | 26     | 35    |
| CG         | 12   | 24     | 36    |

**Table 2: Frequency of Micronuclei in RG and CG**

|        | RG         | CG         |
|--------|------------|------------|
| Mean   | 5.257143   | 4.777778   |
| Standard Error | 0.556615   | 1.011922   |
| Median | 5          | 3.5        |
| Mode   | 5          | 0          |
| Standard Deviation | 3.292977   | 6.071531   |
| Sample Variance   | 10.8437    | 36.86349   |
| Range  | 14         | 25         |
| Minimum| 0          | 0          |
| Maximum| 14         | 25         |
| Sum    | 184        | 172        |
| Count  | 35         | 36         |
| Confidence Level (95.0%) | 1.131177   | 2.054311   |
was statistically insignificant ($P = 0.057$). This could be because patients were already undergoing medical treatment and thereby had lower SLEDAI scores. Few patients had a “high” activity score, which further supports our inference that MN count and disease activity score were both affected by the patients’ current medical treatment regimen.

**Conclusion**

In conclusion, the current study shows that SLE does not lead to a statistically significant increase in the formation of MN in buccal mucosal cells. Thus, proper SLE management may prevent a patient from developing premalignant lesions in the oral cavity.

It will be imperative to pursue a similar study on patients with SLE that have higher disease activity scores, and then determine a correlation with MN frequency. Taken together, from the few studies that have been done on SLE using MN incidence, it may be concluded that the management protocols being utilized at tertiary health care centers have helped significantly to reduce the cytotoxicity of oral mucosae. Assessment of the oral health-related quality of life might therefore be valuable in monitoring the effects of SLE on the oral condition.

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**Conflicts of interest**

There are no conflicts of interest.

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