Influence of genetic polymorphisms on frequency of micronucleated buccal epithelial cells in leukoplakia patients

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SUMMARY

Micronuclei (MN) are extensively used as an indicator of chromosomal damage and early biomarker of cancer risk. The genetic host factors are known to influence the level of chromosomal alterations consequently affecting MN frequencies. Hence, in the present study, we investigated the extent of chromosomal damage by analyzing micronucleated cell (MNC) frequency in exfoliated buccal epithelial cells (BEC) and its possible relationship with genetic polymorphisms in patients with oral leukoplakia (OL). The study group comprised of habit-free (NHC, n = 39), habit controls (HC, n = 62) and OL patients (n = 66). Micronucleus assay was carried out to determine the MNC frequency and the genotyping was performed by PCR–RFLP for metabolizing (CYP1A1, GSTM1, GSTT1, GSTP1) and DNA repair (hOGG1, XRCC1, XPD) genes. The correlation between MNC frequency and genetic polymorphisms was analyzed.

We found significant increase in overall MNC frequency in OL patients as compared to habit-matched controls (p = 0.01). The higher proportion of multiple micronucleated cells (>5 MN per cell) indicate increased DNA damage in the buccal mucosa of OL patients than the controls (p = 0.004). The polymorphic alleles of XPD751 and hOGG1 showed significant association with total MNC frequency in OLs (p = 0.034 and p = 0.03 respectively). In conclusion, the extent of chromosomal damage in target tissues is higher in patients with OL. MNC frequency in combination with genetic polymorphisms in DNA repair genes may serve as better predictor of risk.

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Introduction

Higher incidence of oral squamous cell carcinoma (OSCC) has been reported in South-East Asia and is attributed to the popularity of tobacco habits. 1 OSCC is preceded by a number of precursor stages that induce some morphological changes in cells of oral mucosa, which result in clinically detectable oral pre-malignant lesions (OPLs). The biological behavior of these lesions is rather unpredictable, as some of them may regress, whereas about one-third may progress to invasive malignancy. 2 Our ability to predict the malignant potential of OPLs based on the pathologic findings is limited. Hence, it would be of practical importance to develop biomarkers which will help in identifying individuals at high risk of malignant transformation.

Accumulation of genomic damage leads to genetic instability which may be manifested as chromosomal alterations. The formation of micronuclei in dividing cells is the result of chromosome breakage due to unrepaired or misrepaired DNA lesions or chromosome mal-segregation due to spindle dysfunction. The MN assay is rapid, sensitive, cost-effective and hence extensively used as reliable early biomarker of elevated cancer risk in healthy human subjects. 3,4 The study of DNA damage in exfoliated buccal epithelial cells (BEC) collected from the oral cavity is a minimally invasive method for monitoring populations exposed to genotoxic agents. BEC are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products. About 90% of human cancers originate from epithelial cells. Therefore, oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body. 3,6 We investigated the extent of cytogenetic damage manifested as micronuclei in buccal cells of OLs compared to the matched healthy controls.

The individual response to environmental exposure to genotoxic agents is linked with lifestyle and genetic host factors. Tobacco-specific pro-carcinogens require metabolic activation to cause oxidative DNA damage which is normally corrected by efficient DNA repair machinery. 1 The bioactivation of carcinogens is mediated by phase-I [cytochrome P450s (CYPs)] enzymes and the reactive metabolites generated during the process are conjugated by phase-II [glutathione-S-transferases, (GSTs)] enzymes. Hence,
the coordinated expression and regulation of phase-I and -II enzymes determines the outcome of carcinogen exposure.7 Several cellular repair pathways have evolved as defense mechanisms to maintain the genomic integrity against DNA damage. Nucleotide excision repair (NER) and Base excision repair (BER) systems are multistep enzymatic complexes involved in the DNA repair. Human 8-oxoguanine DNA glycosylase 1 (hOGG1) and X-ray repair cross complementing group 1 (XRCC1) are BER enzymes involved in the core processes of single-strand break repair. hOGG1, a glycosylase, helps in the excision of oxidized guanine, while XRCC1 stimulates endonuclease action and acts as a scaffold protein in the subsequent restoration of the site.8,9 The Xeroderma pigmentosum (XP) genes code for enzymes which initiate NER by sensing and binding lesions and subsequently recruiting the factors to accomplish repair.10 Genes which play vital role in metabolism and DNA repair are polymorphically expressed, with the alleles presenting different enzymatic activities.7,11 Greater health impact and DNA repair are polymorphically expressed, with the alleles modulating cancer risk.11,12 Therefore, the identification of individuals carrying SNPs which alter metabolic capacity and DNA repair efficiency has substantial preventive implications.13 Taking this into account, a further objective in our study was to assess whether the polymorphisms in these genes influence the level of cytogenetic damage. In this paper, we explored the potential associations between various genotypes in genes coding for the CYP1A1, GSTM1, GSTT1, GSTP1, hOGG1, XRCC1 and XPD enzymes and the extent of DNA damage expressed as MN frequencies.

Methods

Study Group

The study group comprised of 66 patients with OLs and 101 healthy control subjects including 62 habit-matched (HC) and 39 habit-free individuals (NHC). All participants completed a detailed questionnaire covering demographic and clinical information along with written informed consent for the enrollment in the present study. The study was approved by the local Ethics Committee.

MN analysis in buccal cells

Exfoliated buccal epithelial cells were obtained from inside of both the cheeks by gently scraping the oral mucosa with a moist wooden spatula and smeared onto the pre-cleaned glass slides. The cells were air-dried, fixed in methanol and stained with 1% Giemsa. To determine the frequency of MNC, a total of 2000 BE cells were examined for each sample. The mean MN frequency in the study groups is summarized in Table 1. The participants were predominantly males (96%). The tobacco habits reported by HC and patients with OLs were categorized as exclusive chewing, exclusive smoking and mixed habits. Age and gender distributions were similar in all the three groups.

Genotype analysis

DNA was isolated from peripheral blood leucocytes collected in EDTA tubes, by Phenol–chloroform DNA extraction method described elsewhere.15 Single Nucleotide Polymorphisms (SNPs) in xenobiotic metabolizing genes, CYP1A1(MspI), GSTM1, GSTT1 (deletion polymorphism), GST P1 (Ile105Val) and DNA repair genes, XRCC1 (Arg194Trp and Arg399Gln), XPD (Lys751Gln and Asp312Asn); hOGG1 (Ser326Cys) were genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) based method described earlier.16–21 The amplified fragments were digested with the appropriate restriction endonuclease enzyme and resolved on 12% polyacrylamide gels followed by ethidium bromide staining for visualization under UV light. Ten percent of the samples were selected randomly for each gene for validation by direct sequencing using automated sequencer (3100 AVANT Genetic Analyzer). Since majority of the NH controls have not given consent to provide blood samples, genotype analysis could not be performed in them.

Statistical methods

The statistical significance for deviation from the Hardy–Weinberg equilibrium (HWE) was determined by χ² test. The mean % MNC frequency in the study groups was analyzed by Kruskal–Wallis and Mann–Whitney test. The subjects were categorized as having high (>5 MN per cell) or low (≤5 MN per cell) MNC frequency on the basis of 75th percentile calculated from the MNC frequency in the control group. Mann–Whitney test was applied to determine genotype frequencies in controls and OLs and to analyze the effect of genotypes on MNC frequency in all the groups. All statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). All p-values were two sided, and probability of less than 0.05 was used as the criterion of significance.

Results

The demographic characteristics of the study population are represented in Table 1. The participants were predominantly males (96%). The tobacco habits reported by HC and patients with OLs were categorized as exclusive chewing, exclusive smoking and mixed habits. Age and gender distributions were similar in all the three groups.

MNC frequency

The results of MNC frequency in the study groups are summarized in Table 2. A stepwise increase in the percentage of micronucleated cells was observed from NHC to HC and from HC to OLs. A significant difference was found between the OLs and HC group regarding the average % MNC frequency (5.70 ± 4.50 versus 4.00 ± 3.38; p = 0.01) but the increase in HC and NHC was not statistically significant (4.00 ± 3.38 versus 2.99 ± 1.74; p = 0.38). In addition, the higher extent of DNA damage as measured by increased frequency of cells containing multiple micronuclei (>5 MN per cell) in OLs than the control subjects with or without tobacco habits was also evident (p = 0.04 and p = 0.004 respectively).

Table 1

<table>
<thead>
<tr>
<th>Demographic characteristics of the study population.</th>
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<tr>
<td>Subjects, n</td>
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<tr>
<td>Age</td>
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<td>Mean ± S.D.</td>
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<td>Median (range)</td>
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<td>Gender, n (%)</td>
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<td>Females</td>
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<td>Tobacco habits, n (%)</td>
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<td>Mixed habits</td>
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