Research Article

Premature Centromere Division in Oral Premalignant and Malignant Patients - A Cytogenetic Biomarker

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Abstract

**Background and Objective:** Premature centromere division (PCD) is characterized by the distinct separation of chromosomes earlier than usual and this cytogenetic phenomenon has been reported to be associated with aneuploidy and cancer. In the present study, PCD test was used to find out whether it could be used as an indicator for oral premalignancy and malignancy.

**Material and Methods:** PCD test was thus used on 60 histopathologically confirmed oral premalignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) patients, using the standard Hungerford method, to find out if this cytogenetic parameter could be used as an indicator for oral disease.

**Results:** The mean of PCD showed an increased chromosomal alteration from controls (5.63 ± 2.67) to OPMDs (23.47 ± 8.08) and further increased mean value in OSCC (29.47 ± 6.29) correlating well with the histopathological diagnosis of the oral disease.

**Conclusion:** The results indicate that PCD can be considered a good and easily identifiable parameter which can be used as a genotoxic biomarker in chromosomal studies, facilitating early diagnosis of oral cancer.

**Keywords:** Tobacco chewers, clastogenic agents, oral premalignant disorders, oral squamous cell carcinoma, chromosomal alterations, genotoxic biomarker.

**INTRODUCTION**

Oral cancer is the 15th commonest cancer in the world and third most common cancer in India. Almost 1,30,000 Indians die due to tobacco related oral cancer.1,2 Tobacco related oral premalignant lesions and oral malignancy arises through an accumulation of genetic alterations, which includes chromosomal alterations, DNA changes and/or epigenetic alterations. Many cases of oral malignancy develop from premalignant lesions or conditions of the oral cavity.3 Only early detection, histopathological investigation, genetic tests, and treating tobacco related oral cancer patients especially in their premalignant state are the only hope in reducing the burden of this disease. Even though the last five decades have witnessed the introduction of a number of relatively rapid genetic tests for detecting tobacco related oral cancer, a simple yet a sensitive test for early detection seems to be the need of the hour. One such cytogenetic test is the Premature Centromere Division (PCD).

PCD is a phenomenon of the loss of control over sequential separation and segregation of chromosome centromeres, characterized by the distinct separation of chromosomes earlier than usual.4-8 Vig proposed a hypothesis that PCD may result in non-disjunction by impairing the attachment of prematurely separated centromeres to spindle fibers.9 In a four dimensional cascade of cyclin regulated cell cycle kinetics, chromosomes seem to have an important role in sustaining the steadiness of the fluctuating genome. Abundant data analysis shows that...
the spatial organization of chromosomes is usually accompanied by the sequential regulation of replication and the separation and segregation of these processes in dividing cells.\textsuperscript{10-14} Centromere separation takes place in an orderly manner in which certain chromosomes tend to separate first. In humans the first chromosome to separate is chromosome 18. In an ordered sequence, chromosomes 17, 2, 10, and 12 follow the separation of chromosome 18.\textsuperscript{15-17} This sequence of chromosome separation is capable of becoming deregulated in ageing cells, Alzheimer's disease and various tumors and chromosome instability syndromes.\textsuperscript{18-22} Recent investigations have established the fact that PCD yields are significantly higher in populations exposed to mixed chemicals, pesticides, crude oils and cytostatic drug.\textsuperscript{23-26} Centromere instability has been recognized as a hallmark of human cancer caused by continuous mis-segregation during mitosis in which errors of centromere separation, particularly PCD, is seen to have an important fundamental role.\textsuperscript{27-30} PCD is at present a well-recognized phenomenon associated with aneuploidy and cancer.\textsuperscript{31-34}

Thus in the present study, PCD test was used on histopathologically confirmed oral premalignant and malignant patients, to find out if this cytogenetic parameter could be used as an indicator for oral disease. Also to utilize the findings to facilitate early diagnosis and subsequent disease management tailored to the individual patient lessening the mortality and morbidity associated with the disease.

**MATERIAL AND METHODS**

This is a human descriptive and analytical study, conducted at Mahatma Gandhi Medical College and Research Institute (MGMCR1), Pondicherry, India, a rural tertiary care hospital. The study was designed in accordance with the Helsinki II declaration and approved by the Institutional Human Ethical Committee.

**Subject recruitment and sample**

Patients referred from the clinical departments of ENT, TBCD and General Surgery of MGMCR1, and also from Oral Medicine and Oral & Maxillofacial Surgery departments of Indira Gandhi Institute of Dental Sciences (IDIGS), Pondicherry, India, to the department of Pathology, MGMCR1 with suspected cases of oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) were included in the study. After due examination of the signs and symptoms of the OPMDs and OSCC, tissue biopsy was taken and processed for histopathological slide preparations. Two ml of fresh peripheral blood was collected from OPMDs and OSCC patients and control subjects, under sterile conditions by venipuncture into heparinized syringes for cytogenetic study. Lymphocyte cultures were set up from the heparinized blood according to the method of Hungerford, with minor modifications by the addition of cold fixative after hypotonic treatment.\textsuperscript{35} The time of blood sampling was identical to that of the patient and the control.

**Data collection**

Signing an informed consent and reply to a questionnaire elaborated to determine the profile and habits of the study population from the subjects and controls were taken at the time of taking tissue biopsy and blood collection. Each subject was asked about his/her lifestyle, food consumption, infectious diseases, X-ray exposure, medication during the last three months at the time of investigation, etc. Age- and sex matched control group comprised of normal healthy individuals belonging to the same socioeconomic group, lifestyle and dietary habits who did not have the habit of taking pan masala, areca nut or tobacco in any form, chewing or smoking including passive smoking and alcohol consumption. The parameters monitored were Histopathological types, Histopathological grading and for Premature Centromere Division

**Histopathological study**

Systematic examination of all cases of oral biopsies of OPMDs and OSCC conditions received in Pathology Department of MGMCR1 from December 2013 to December 2016 were analyzed with reference to clinical features, gross features and the biopsy specimens were processed. Paraffin blocks were made, then sectioned and stained with hematoxylin and eosin. A detailed microscopic examination was done using light microscope and the findings were recorded with special emphasis on the different histopathological OPMDs, and grades of OSCC. By the histopathological examination of slides, OPMDs were classified as per the guidelines laid down by WHO 2005 (Oral Leukoplakia and Oral Sub mucosal Fibrosis), while OSCC were classified as per Broders’ grading system into well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma.\textsuperscript{36,37}
Cytogenetic study

The cytogenetic study involved two groups consisting of 60 OPMDs and OSCC patients and 60 age- and sex matched control group. Only histopathologically confirmed patients of OPMDs and OSCC were included in the cytogenetic study while patients with chromosomal anomalies like Klinefelter’s (47XXY), Klinefelter’s Variants (47XYY), Male Turners Mosaics (46XY/45XO), Turner Syndromes (45XO) and OPMDs and OSCC patients undergoing radiation treatment were excluded from the study.

RESULTS

Out of 60 cases of OPMDs and OSCC, 41 were males and 19 were females in a ratio of 2.16 male: 1 female with a mean age 58.7 ± 12.7 years. Fifteen cases were histopathological diagnosed with OPMDs (8 Oral Leukoplakia - OLKP and 7 Oral Sub mucosal Fibrosis - OSMF while 45 cases were diagnosed with OSCC (12 well differentiated- WDSCC, 24 moderately differentiated- MDSCC and 9 poorly differentiated squamous cell carcinoma -PDSCC). All cases in the study group belonged to tobacco related oral cancer who were in the habit of taking betel quid ingredients consisting of betel leaf, areca nut, slaked lime, and sun-dried tobacco.

The mean Premature Centromere Division (PCD) showed a significant difference between the study groups when the data were analyzed using ANOVA and student’s t test. P value <0.05 was considered as significant. PCD was significantly different between the controls, OPMDs & OSCC cases. There was a significant difference between OPMDs & OSCC also (Table 1). The mean of PCD showed an increased chromosomal alteration from controls (5.63 ± 2.67) to OPMDs (23.47 ± 8.08) and further increased mean value in OSCC (29.47 ± 6.29) correlating well with the histopathological diagnosis of the oral disease (Figure 1).

Table 1: Comparison of PCDs in study group

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean PCD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60</td>
<td>5.63 ± 2.67</td>
</tr>
<tr>
<td>OPMDs</td>
<td>15</td>
<td>23.47 ± 8.08a</td>
</tr>
<tr>
<td>OSCC</td>
<td>45</td>
<td>29.47 ± 6.29bc</td>
</tr>
</tbody>
</table>

* ANOVA shows significant p-value for all groups of study
  a p<0.001 when compared to control
  b p<0.001 between OPMDs & OSCC cases
DISCUSSION

During the process of mitosis, microtubules are formed at the prophase stage and have the kinetochores of the chromosomes attached to it in the metaphase stage. Metaphase stage has condensed and highly coiled chromosomes, align in the equatorial plate of the cell. Anaphase stage is the pulling of chromatids by the microtubules to the poles and is accomplished by regulation of the anaphase-promoting complex, securin, and separase. Securin is a protein involved in control of the metaphase-anaphase transition and anaphase onset. Securin, produces an abrupt stimulus that induces highly synchronous chromosome separation in anaphase in coordination with Separase, a cysteine protease that regulates the separation of sister chromatids during the early stage of anaphase. PCD is thus a phenomenon of the loss of control over sequential separation and segregation of chromosome centromeres during mitosis, characterized by the distinct separation of chromosomes earlier than usual. PCD is usually diagnosed when a separation between the sister chromatids was equal to or more than thickness of chromatid. In humans, during the metaphase-anaphase transition, the centromeres of chromosomes number 2, 8, 17, and 18 separate earlier, while chromosomes 13, 14, 15, 21, and 22 are the last to split into two subunits. But, during the leukocyte cultures of the present study on tobacco related oral cancer cases, we identified PCD, even though the cells were arrested at metaphase using a mitotic inhibitor, colchicine such that all the chromosomes analyzed should have been seen with the two chromatid held together by the centromere. PCD indicates that the genotoxicity has played a role such that the kinetochore had failed to attach properly to the microtubules generating a premature progression of chromatids, a condition of anaphase transition that are easily seen in the metaphase analysis of chromosomes. In such condition, the anaphase-promoting complex, Securin and separase seem to have lost its highly synchronous chromosome separation process. Thus PCD indicates a parameter of chromosomal instability which may lead to mitotic checkpoint abrogation and aneuploidy.

The Premature centromere division (PCD) in the present study showed a significant p-value between the controls, OPMDs & OSCC cases through ANOVA. PCD also showed a significant p-value through t-test observed between the pairs of group namely the controls & OPMDs cases, between the controls & OSCC cases and also between the OPMDs & OSCC cases (Table 1). The results clearly indicate that the genotoxic effects of tobacco and its related clastogenic agents in the betel quid has caused the chromosomes to separate prematurely. Thus PCD can be considered a good and easily identifiable parameter which can be used as a genotoxic biomarker in chromosomal studies. The increased mean value of PCD of the controls and between OPMDs and different grades of OSCC correlates well with the histopathological diagnosis of
the disease (Figure 1). The result shows that the mean PCD count in OSMF was 23.4 while in OLKP it was 23.5, signifying that the mean value of 23.45±0.07 can be taken as a value indicative of premalignant condition, such that it may help in preventing such patients from falling a prey to malignant condition of oral squamous cell carcinoma, making the parameter PCD, a valuable indicator for cytogenetic damage.

CONCLUSION

Centromere instability has been recognized as a hallmark of human cancer caused by continuous mis-segregation during mitosis in which errors of centromere separation, particularly PCD, is considered a cytogenetic predictor as it is at present a well-recognized phenomenon associated with aneuploidy. Major et al have suggested that PCD can be developed into a new, all types of exposure-related cytogenetic biomarker for a more adequate occupational cancer risk assessment. 42, 43 Thus, the applicability of PCD test as a biomarker for monitoring studies in human populations exposed to various genotoxic agents should remain open as the method is easy, and takes lesser time and does not require expertise.27-29

CONFLICTS OF INTEREST

None.

REFERENCES

A new study published in The Journal of Sports Medicine and Physical Fitness aimed to examine whether 500-m and 1-km moderate treadmill-walking test (TWT) equally estimate VO2peak in male outpatients with cardiovascular disease (CVD). Here, 142 clinically stable male outpatients with CVD, aged between 34 and 92 years performed a moderate and perceptually-regulated 1k-TWT.

The results showed that VO2peak estimated from the 500-m test was not different from that estimated from the 1k test, in these patients. The coefficient of correlation between the two was deduced as 0.98; while the slope and the intercept of the relationship between the 500-m and 1k tests were not different from the line of identity. Furthermore, Bland-Altman analysis demonstrated that 96% of the data points were within two standard deviations.

It was concluded that 500-m treadmill-walking test, although a shorter version of the original 1k test, is a reliable method for estimating VO2peak in stable male outpatients with CVD and is more time efficient. It was stated that these findings have practical implications in the context of transitioning patients from clinically based and supervised programs to fitness facilities or self-guided exercise programs.