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A STUDY OF DNA DAMAGE IN THE SALIVA OF ORAL SQUAMOUS CELL CARCINOMA

Biochemistry

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ABSTRACT

Oral cancer is a sub-type of head and neck cancer, which is any cancerous tissue growth located in the oral cavity. India has highest incidence of OSCC in the world. The development of human cancer is depending on several factors such as DNA damage and DNA repair system. SCGE is used for measuring low level of strand breaks with high sensitivity in cells. The study consists of 115 OSCC patients and 45 control subjects. This study found that, as the severity of OSCC increases, the DNA damage proportionally increases which is marked by MTM. The increased level of MTM significantly in OSCC patient than the healthy controls, indicates that severe DNA damage occurs in OSCC patients during disease progression.

KEYWORDS

OSCC, SCGE, MTM, DNA damage, DNA repair.

Introduction

Cancer is the second leading cause of death, worldwide. Approximately eleven million new cases of different types of cancer are diagnosed every year (1). Various types of cancer are associated with heterogeneous mutation, in different types of genes as a result of malignancy, out of which squamous cell carcinoma (SCC) is considered as the largest one (2-3). Oral cancer is a sub-type of head and neck cancer, which is any cancerous tissue growth located in the oral cavity (4). There are several types of oral cancers, but around 90% are oral squamous cell carcinoma (OSCC), which originate in the tissues that line in the mouth and lips. OSCC involves in tongue, floor of the mouth, cheek lining, gingiva (gums); lips and palate (roof of the mouth). OSCC is the sixth most common human cancer, with an increasing incidence in younger people with high morbidity rate (5-6). India has highest incidence of OSCC in the world. Around 50-70% of cancer related death in India are due to oral cancer (7-8). According to histological findings, OSCC are graded into three distinct categories, by Border's grading system

Grade 1: Well differentiated oral squamous cell carcinoma (WD OSCC)
Grade 2: Moderately differentiated oral squamous cell carcinoma (MD OSCC)
Grade 3: Poorly differentiated oral squamous cell carcinoma (PD OSCC)

The severity of OSCC by the grading system is according to descending order.

The development of human cancer is multifactorial and depends on several factors; such as DNA damage, effectiveness of antioxidant defense, DNA repair system and level of reactive oxygen species (9). Saliva is a clear, slightly acidic (pH = 6.0–7.0) and complex biological fluid composed of secretions from major salivary glands: the parotid, sub-mandibular, and sublingual glands, as well as multitudes of minor glands including labial, buccal, lingual, and palatal tissues. Wu et al recentlysuggested that saliva play a pivotal role in OSCC pathogenesis and demonstrated that saliva play an important role in cigarette related nicotine induced DNA damage (10-11).

Materials and method

The study population consisted of 115 OSCC patients. OSCC was diagnosed clinically and histopathological during their visit to the Maitri college of dentistry and research center Durg. The control population consisted of 45, apparently healthy, age and sex matched subjects. All the subjects were informed about the procedure and study was carried out after their consent.

Saliva sample collection

Unstimulated whole saliva samples were collected, after their histopathological conformation of the clinical diagnosis of OSCC, between 10 a.m. and 12 p.m., two hours after the subject's usual breakfast time. The subjects were asked to rinse the mouth with distilled water thoroughly to remove any food debris and then to spit into a sterile small plastic container. Once the saliva (2 ml) was collected, the plastic container was placed in ice carrier box and transferred to the laboratory for biochemical analysis (12).

Single Cell Gel Electrophoresis (SCGE)

The Geno toxicity was measured in saliva by single cell gel electrophoresis (SCGE), commonly known as comet assay, by Singh et al (13). Comet assay is the simple biochemical technique for detection of DNA single-strand breaks, alkali-labile sites, and incomplete excision repair sites. This method is used for measuring low level of strand breaks with high sensitivity in cells. A damage Cell in the comet assay has the appearance of comet with brightly fluorescent head and a tail whose fluorescent intensity is related to the number of strand breaks present. Cells with undamaged DNA will appears as intact comet head without tails after specified electrophoresis time.

5ul of whole saliva were suspended in 0.5%low melting agarose. It is then sandwiched between top layer of 0.6% normal melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. After solidification of agarose layer, the slides were immersed in lysis solution at 4°C. After 30 minutes, slides were placed in electrophoresis buffer for 30 minutes at room temperature. The electrophoresis buffer was then chilled to perform electrophoresis at 250mA and 15V in horizontal electrophoresis for 30minutes. After electrophoresis slides were neutralizes by Tris-HCL buffer and stained with ethidium bromide. Slides were analyzed using epifluorescence microscope. DNA damage was classified by visual classification and categories in to comet depending upon amount of DNA in the tail (14).

Statistical Analysis

The relationship between the frequencies of changes in DNA damage in saliva of OSCC patients as compared to control were assessed by using graph pad prism software (version 4.2). Differences with the values of $P < 0.05$ were considered to be statistically significant.

Observations and Result

In this study the DNA damage is shown by single cell gel electrophoresis (SCGE) that is by comet assay. The result of SCGE (comet assay) shows a DNA damage in OSCC patients compare to healthy controls. Upon staining cellular DNA is measured for fluorescence with a microscopy imaging system. The resulting image resembles a “comet”, with the cellular DNA segregating into a “head” and “tail”. The head is composed of largely intact genomic DNA, while the tail comprises damaged (SSBs, DSBs) or fragmented DNA, with the fluorescence intensity and length of the tail being directly proportional to the extent of the DNA damage. In the normal (control)

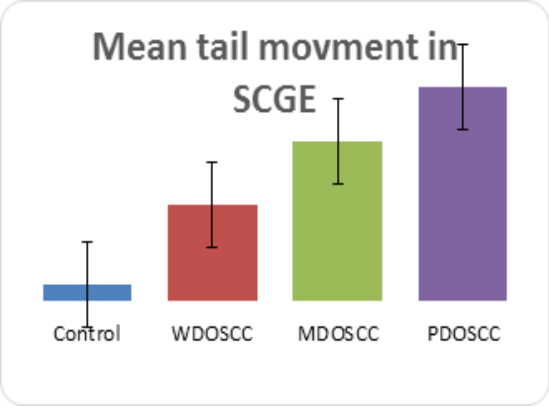
shown in Figure-1, there is intact head formation and no formation of the tail. This show that there is no damage of DNA in control subjects. In Figure-2, slightly formation of the tail occurs in WDOSCC which shows less damage of DNA content. In MDOSCC, moderate formation of tail shown in Figure-3 which indicate that moderate amount of DNA damage occur in this condition. The most severe condition is PDOSCC, where maximum amount of DNA damage takes place. In the Figure-4, scattered tail formation which indicates the severity in this condition.

The values of MTM (mean tail movement) were more in OSCC patients than healthy controls. The values are increases with the severity of the disease. MTM value for control is 0.08; for WDOSCC is 0.49; for MDOSCC is 0.81 and for PDOSCC is 1.09. These values show the level of DNA damage. As the severity increases the DNA damage is also increases. The values are given in Table and shown in Graph. The values are statistically significant.

Table: Mean tail movement in SCGE of OSCC patients as compared to control

	MTM (mean tail movement)
Control (n=45)	0.08±0.001
WDOSCC (n=65)	0.49±0.16 *
MDOSCC (n=35)	0.81±0.43 **
PDOSCC (n=15)	1.09±0.40 **

*P<0.01
**P<0.001



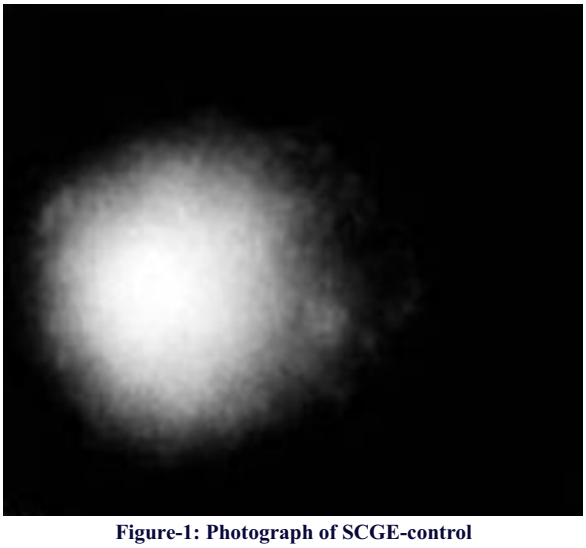


Figure-1: Photograph of SCGE-control

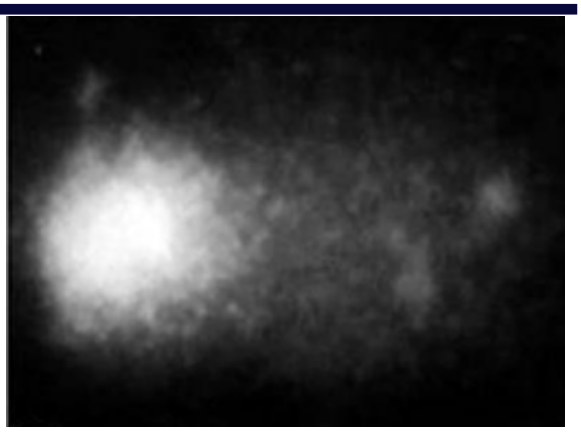


Figure-2: Photograph of SCGE-WDOSCC

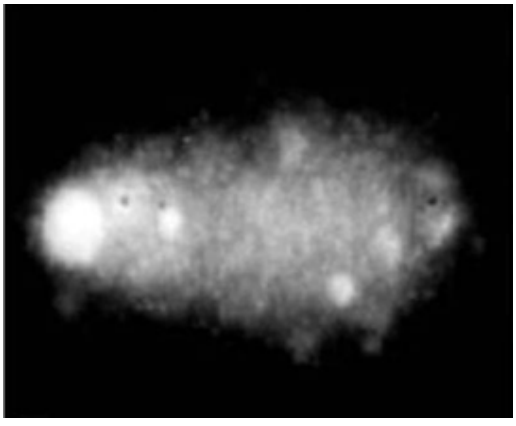


Figure-3: Photograph of SCGE-MDOSCC

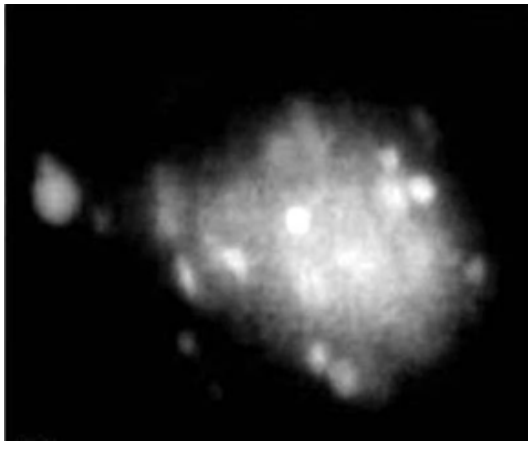


Figure-4: Photograph of SCGE-PDOSCC

Discussion

OSCC is the sixth most common malignancy and one of the major causes of cancer morbidity and mortality in the world. About 50,000 patients are diagnosed with oral and pharyngeal cancers every year, out of those, three quarters of patients are from the developing countries (15).

Cancer is essentially an event occurring at the gene level where DNA damage ultimately resulting into carcinogenesis. However, multiple factors such as viruses, chemicals, irradiation and the genetic makeup of the individual can also contribute to carcinogenesis.

Saliva can be used for detection of oral malignancy and pre-malignancy because of anatomical proximity to the lesions. The squamae's and cells exfoliated from lesion tissue can be easily tested cytological for OSCC (16).

In order to examine oxidative DNA damage during OSCC, single cell gel electrophoresis (SCGE) was performed. It is also called comet assay is a sensitive method to detect DNA single strand break and alkali-labile site (17-19). This study found that, as the severity of OSCC increases, the DNA damage proportionally increases which is marked by MTM (mean tail length). In some studies, such type of findings was observed in the previous research (20-21).

Conclusion

saliva has been considered as the vital body fluid and mirror image of blood for a long time to investigate biomarkers for early detection of diseases. The various components of saliva acts as a mirror of body health. Since the ancient times, saliva is being used for detection of various diseases ranging from autoimmune diseases to infections including different type of cancers.

According to MTM (mean tail movement), there was increased level of MTM significantly in OSCC patient than the healthy controls. This indicates that severe DNA damage occurs in OSCC patients during disease progression.

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