ORIGINAL RESEARCH PAPER

Volume-8 | Issue-3 | March-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE STUDY OF DNA DAMAGE IN TOBACCO CHEWERS AND HEALTHY INDIVIDUALS BY COMET ASSAY

Anatomy

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ABSTRACT

Tobacco is either chewed or smoked; it is equally harmful in both forms and there is no safe way to use the tobacco. Use of tobacco is now emerging as one of the major factors responsible for premature deaths in India. Comet assay is performed under alkali conditions and it can estimate damage in a single cell. The result of the present study indicates the greatest amount of damage in tobacco chewers as compared to controls. Comet assay is a valuable tool to assess the DNA damage.

KEYWORDS

Tobacco, comet assay, DNA damage

INTRODUCTION:

Tobacco is an agricultural product that belongs to the family Solanaceae in the genus *Nicotiana.* Tobacco appears as old as human civilization. During the Mughal era, smoking of tobacco in the form of hukka was a famous royal habit. Smokeless tobacco contains tobacco, which is either chewed, sucked or inhaled. The data collected through GATS (Global Adult Tobacco Survey) India-2 in 2016-17 revealed that 28.6% adults (15 years and above) use tobacco, i.e. 26.7 crores. Among them, 21.4% adult uses smokeless tobacco, which is less from GATS India-1in 2009-10. The mean age of initiation of use of tobacco also shows increase of one year, i.e. 18.9 years. [1] Tobacco is either chewed or smoked; it is equally harmful in both forms and there is no safe way to use the tobacco. Use of tobacco is now emerging as one of the major factors responsible for premature deaths in India.[2]

DNA is a Key molecule as it stores all the genetic information needed for the development of human beings. Damage to the DNA generate the lesions on it and if a base is altered, then it promotes breaks in the helix of DNA.[3] The breaks in double strand are lethal to the cells due to loss of genetic information. Comet assay is known as comet assay because of the appearance of damaged DNA like a comet having a head (intact DNA) and a tail (fragments of DNA). This method is also named as single cell gel electrophoresis (SCGE) as it can estimate DNA damage in the individual cell.[4] This assay is performed under alkali conditions (pH >13) so, called as an alkaline comet assay. Under the fluorescent microscope, the DNA of undamaged cell looks as a bright circular mass lying in the cavity of the lysed cell. While in the damaged cell; fragments of DNA looks like a tail streaming from this bright circular mass. The Damage to the DNA can be calculated through many parameters such as %head DNA, tail length (TL), %tail DNA, tail moment (TM) and olive tail moment (OTM).

Objectives:

The aim of the present study was to assess the prospective of comet assay as a biomarker for DNA damage in tobacco chewers.

MATERIAL AND METHOD:

The present study was conducted on 50 control subjects that were not having any habits of chewing tobacco and 58 tobacco chewers. The subjects chosen for the present study were the patients visiting to OPD of National Institute of Medical Sciences & Research and Hospital (Jaipur). The study was approved by the Institutional Ethical Committee. The subjects were informed about the study and a written consent was obtained from them. The age of the subjects ranged from 21 years to 75 years. Comet assay was performed on whole blood under alkali conditions. The ethidium bromide was used for staining and analyzation of the slides. DNA (ethidium bromide) in lymphocyte cells emits reddish color. 100 cells for each subject were chosen randomly. The photographs were taken and analyzed by comet score software. The following comet parameters were used in the study-

- **% DNA in head-** Percentage of amount of DNA present in the head.
- **Tail length (TL) -** Length of the tail of the comet is measured from the right border of the head area to the end of the tail.
- **% DNA in tail-** Percentage of amount of DNA present in the tail.
- **Tail moment (TM) -** It is calculated by multiplication of % tail DNA and tail length.
- **Olive tail moment (OTM) -** It is calculated by combining two aspects of comet, i.e. shape and intensity. Its value can be obtained by the following formula-

OTM =% tail DNA x (tail CoG–head CoG)

Where, tail CoG= Centre of gravity of the DNA into the tail Head CoG= Centre of gravity of the DNA into the head One way ANOVA analysis was used to determine the significance of the different parameters measured between the controls and tobacco chewers. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS:

In controls, the mean ± SE of % head DNA was 89.36 ± 1.09 and in tobacco chewers, it was 86.28 ± 1.98, which indicates the DNA damage in the chewers. In controls, the mean ± SE of tail length (TL) was 1.84 ± 0.23 and in tobacco chewers it was 5.51 ± 0.61. The difference in % head DNA was although non-significant, but the difference in tail length was highly significant. Along with these two parameters the other parameters like % tail DNA, tail moment and olive tail moment was also more in tobacco chewers. The data of the present study clearly indicate that the comet assay can be used effectively for prediction of DNA damage in chewers.

Table 1: Comparison of Mean ± SE of DNA damage assessment in blood by alkaline comet assay among tobacco chewers and controls

DNA damage assessment	Control	Tobacco chewers	P value
% Head DNA	89.36 ± 1.09	86.28 ± 1.98	0.20 (NS)
Tail length (TL)	1.84 ± 0.23	5.51 ± 0.61	<0.001 (HS)
% Tail DNA	10.63 ± 1.09	13.71 ± 1.98	0.20 (NS)
Tail moment (TM)	0.26 ± 0.05	0.95 ± 0.23	0.01 (S)
Olive tail moment (OTM)	1.28 ± 0.16	1.46 ± 0.20	0.51 (NS)

DISCUSSION:

The results similar to our present study were found by **Manikantan P et al.**[5] (2010). A total of 76 subjects out of which 38 were tobacco users and 38 controls were assessed. To assess the DNA damage they have measured mean tail moment (MTM). In tobacco chewers mean tail moment was 0.68 ± 0.29, while in controls it was 0.35 ± 0.42. They found a significant difference in the mean tail moment of controls and chewers and reported that in comparison to controls DNA damage was more in chewers.

Guttikonda VR et al.[6] in 2014 conducted a study in peripheral blood leukocytes by comet assay. They selected 30 controls, thirty patients with tobacco habituation. They found that mean DNA damage (μm) in controls was 2.18, while in tobacco users it was 2.58. Similar to the

present study, they also concluded that DNA damage is more in tobacco users.

Very less number of studies have been conducted in tobacco chewers and only few of them has used the comet assay. Till date, there is no baseline frequency to assess the damage by comet assay. So there is a strong need of a large population study for interpretation of results.

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