

viii. An abstract, describing the background, objectives, methodology of the Proposed project:

Most of the environmental agents are proved to be genotoxic and lethal to the DNA; which causes strand breaks. If such strand breaks persist i.e., are not rectified by the inborn mechanism of human body, it may leads to genetic mutations and carcinogenesis; i.e., generation of DNA damage is considered to be the initial event in many pathological conditions. Comet assay or single cell gel electrophoresis assay is a simple and cheap method to detect the level of DNA damage at cellular level. Benzene, one of the components of Petrol, is a hazardous agent and has been classified as a human carcinogen by the International Agency for Research and Cancer (IARC, 1989). The objective of the present study is to detect the level of DNA damage by comet assay, in people who are exposed to Petrol vapor and comparing the same with the healthy controls of same age group. Mononuclear cells from the blood sample of these persons will be subjected for electrophoresis after the lysis of cell membrane. The damaged DNA strands will be migrated out from the cells and give the appearance of the comet. Quantifying the amount of DNA in the tail gives the level of DNA damage.

ix. Details of the project proposal including the state-of-art of the subject, the work already done in this area in India/abroad and defining clearly the objectives and methodology and year Phasing of the project:

Introduction

Many number of assays are exists for the detection of genotoxic effect of various compounds but many in-vivo, ex-vivo and in vitro studies proved that single cell gel electrophoresis or comet assay (Singh et al., 1988), is technically simple, fast and cheap method to detect DNA damage from any type of mammalian cell (Collins AR, 2009). Genotoxic effects of Petroleum products and other aromatic hydrocarbons can be detected through this method (Pitarque et al., 1997, Roma-Torres et al 2006, Keretsetse et al, 2008).

The purpose of the present study is to measure the level of DNA damage in persons who are exposed to Petrol/Diesel vapor and to compare the values with that of healthy controls of same age group.

Methodology

Petrol pump attendants of Puducherry union territory (excluding Mahe, Yanam & Karaikkal) are included in the study. Healthy subjects of same age and sex will be selected from the same area as control group. The study will be executed after the approval of Institute Research council & Human Ethics committee.

Comet assay will be conducted as per the standard protocol (Ahuja & Saran, 1999). For the purpose of comet assay 5 ml of venous blood is collected from each individual and lymphocytes are separated using Histopaque®. The cells thus obtained are mixed with melted low-melting-point agarose and layered on normal-melting-point agarose-precoated microscope slides and allow to solidify. A third layer of normal-melting-point agarose will be added on top of the cell-containing layer, and allow to solidify. These slides are then treated with membrane lysing solution to enable the nucleoid to be free. In the next step slides are subjected for electrophoresis; so that the fragmented/damaged DNA will get migrated from each cell towards the anode and thus created the "comet" like shape. After electrophoresis the slides are fixed, dried and stained using Silver nitrate solution. Stained slides will be observed with Trinocular research microscope with camera attachment. From each slide 25 to 50 randomly selected cells will be photographed and used for comet scoring to detect the damaged DNA in each cell. Difference in value of comet parameters between the groups will be analysed using standard statistical tests. If the level of DNA damage is more in persons who are exposed to Petrol vapor than the control people, it is considered significant.

References:

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