MATERIALS AND METHODOLOGY

SOURCE OF DATA

This study would be conducted in the Department of Conservative dentistry and endodontics, Rama dental college hospital and research center Kanpur.

The subjects examined in this study will be patients in the age group 30-70 years with dental caries and may or may not be known diabetic patients undergoing treatment for this disease.

Before the study is conducted, permission will be taken from the ethical committee of institution and a written consent will be obtained from all the subjects participating in the study.

All the subjects participating in the study will be divided into two groups.

GROUP-I: PATIENTS WITH TYPE II DIABETES MELLITUS WITH DENTAL CARIES;

GROUP II NON DIABETIC PATIENTS EXPERIENCING DENTAL CARIES.

The individuals included in GROUP I should be known Type II diabetes mellitus patients undergoing treatment for the last five years. Information regarding the past medical history of each individual would be obtained in order to rule out if the patients are suffering from systemic diseases other than Type II diabetes mellitus. Individuals who are diagnosed to be suffering from other diseases will be excluded from the study. A written consent will be obtained from the individuals. After that a questionnaire will be given to the patient to obtain information regarding duration of the disease and the type of treatment received for the disease.

Levels of control of TypeII diabetes mellitus will be assessed by recording lab findings such as RBS.

Level of caries risk among these type II diabetic patients will be assessed by caries assessment management by risk assessment (CAMBRA). The CAMBRA proforma gives us the essential information regarding the fluoride exposure, sugar intake and caries experience of mothers. This information aids in categorizing the patients into low, medium and high risk patients thus providing valuable suggestions in preventing caries.
Individuals included in GROUP II will be non diabetic patients with dental caries.

**MEASUREMENT OF SWEET PERCEPTION.**

A questionnaire containing a list of items will be given to all the subjects participating in the study. Further they will be asked to rate their liking for food which will be evaluated by using a 9-point scale ranging from like extremely to dislike extremely. An option of never tried this food will also be included in the questionnaire, and the study. The mean of liking that will be given by each individual to the foods will give us the sweet liking score. The subjects who had never tried the sweet foods included in the questionnaire will be excluded from

**SALIVARY TESTS**

In all the patients’ clinical examination of the oral cavity will be performed by a single investigator who will be blinded to the genotype of the subjects participating in the study. To stimulate the salivary flow in all the subjects, they will be asked to chew a standard paraffin wax tablet and the volume of the saliva collected averaged for five minutes, would give the desired stimulated salivary rate. Bacteriological counts for the lactobacillus and mutants streptococcus will be calculated by culturing on bacteria specific, sugar containing agar (MSB media and Rugosa agar from VWR Canada). These salivary samples will be incubated at 37°C for 48 hours.

**ORAL HYGIENE ASSESSMENT**

A questionnaire which gathers information regarding the frequency of brushing, the various oral hygiene aids used and the factors causing the caries risk will be given to all the subjects of this study. Oral hygiene of all the subjects will be evaluated by using the Leo and Silness indices, which provide information about the plaque and gingivitis score.
SUGAR INTAKE ASSESSMENT

The simple sugar consumption in each individual is assessed by interviewing them about their detailed dietary history. The individuals will be interviewed about the average intake of food consumed by them daily, the usual choices of food. A questionnaire will be given to collect information regarding their current oral hygiene practices including the frequency of brushing and oral hygiene aids used and existing caries risk factors (e.g. Fluoride exposure). A prospective 3 day dietary record would be collected. The frequency of daily sugar exposure was calculated based on exposure to any sugar containing foods at or between meals. A caries risk assessment score was tabulated per individual, based on a caries risk questionnaire, incorporating the data on daily sugar exposure. All forms for data collection can be found in drinks routinely preferred during major meals of a day.

DENTAL CARIES ASSESSMENT

With the aid of an air syringe, mouth mirror and probe the occurrence of carious lesions in the oral cavity of each individual will be assessed. Three different indexing systems will be conducted to assess the dental caries. The first indexing system is the DMFT (decayed, missing, and filled) score which will be used to measure the prevalence of dental caries in the individuals and it will be recorded for each patient according to the recommendations of WHO for epidemiological surveys and it is applicable for the overall number of teeth present in the mouth. The second indexing system uses a combination of DMFT index with radiographic findings. While using this system a tooth is considered to be missing only when it is extracted due to caries. ICDAS is the third indexing system that will be used in this study to detect, assess, diagnose and monitor caries. According to the ICDAS protocol the patient has to follow certain instructions for oral prophylaxis, then the wet tooth surfaces will be examined for surface contour, minor cavitations or sealants.

International Caries Detection and Assessment System

Decision Number 2: classification of the carious status based on ICDAS

Sound tooth surface: Code 0
No evidence of caries

**First visual change in enamel: Code 1**
Surface discolouration evident on prolonged drying

**Distinct visual change in enamel: Code 2**
Carious opacity or brown discoloration evident when tooth surface is wet.

**Localized enamel breakdown because of caries with no visible dentine or underlying shadow: Code 3**
Loss of tooth structure is evident on drying

**Underlying dark shadow from dentine: Code 4**
This lesion appears as a shadow of localized breakdown

**Distinct cavity with visible dentine: Code 5**
Cavitation in opaque or discoloured enamel exposing the dentine beneath.

**Extensive distinct cavity with visible dentine: Code 6**
Extensive carious lesions involving half of tooth structure

**GENOTYPING**

For genetic analysis DNA will be isolated from the buccal epithelial cells of suspected persons that will be obtained by buccal swabs of all the individuals participating in the study. The DNA will be extracted instructions using a commercial kit (Genome diagnostic). The SNPS of TAS1R2 and GLUT2 genes would be genotypes. After the isolation of DNA we have to set the optimum conditions of PCR for the amplification of these genes. Then the amount of DNA would be checked with agarose gel electrophoresis.
SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

Sample size has been calculated in order to control type I and type II error. Assuming a minimum power 80% and 95% significance level the sample size has been calculated using this formula:

\[
n = \frac{2(P)(1 - P) (Z_\beta + Z_{\alpha/2})^2}{d^2}
\]

\( p = \) incidence of Type II diabetes mellitus

\( q = (1-p) \)

\((P1-P2)^2 \) or \( d^2 \) is the difference which we want to detect at a specified power and level of confidence.

\( Z_\beta \) – power of statistical test we want to be minimum 80% for which is \( Z_\beta \) is 0.84.

\( Z_{\alpha/2} \) is the level of confidence we have chosen 95% confidence in this \( Z_{\alpha/2} = 1.96. \)

When \( p \) indicates Type II diabetes mellitus

According to the literature the incidence of diabetes mellitus is 12.1%

Then \( q = (1-p) = 0.879 \)

Setting the allowable error \( d \) to be 10%

The calculated sample size comes for our study is 83

In order to control loss of follow up and manual errors for which we rounded the sample size of 100 for each group.
Data will be collected and entered in MS excel work sheets and results will be analyzed with appropriate statistical tool like tests of significance, logistic regression analysis etc using SPSS version software.