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Research Article

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Genomic wide DNA methylation analysis in blood of local population: A survey in Southern India

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ABSTRACT

Epigenetics is the study of modification in gene expression that does not involve changes in DNA nucleotide sequences. DNA methylation is an epigenetic mechanism that offers a great potential for identification of biomarkers to detect and diagnosis cancer in its earlier stage and to accurately assess individual risk. The current study sought to investigate genome wide methylation in blood of 55 volunteers. The volunteers were questioned to analyse the relationship of global DNA methylation pattern with their age, family history of cancer, diet, alcohol intake and smoking habits.

Keywords: Epigenetics, Methylation, Cancer

INTRODUCTION

Cancer, medically known as malignant neoplasm, is a group of more than 200 diseases characterized by uncontrolled proliferation, colonisation and spread of abnormal cells to different parts of the body through the blood stream and lymphatic system [1, 2, 3]. A neoplasm or tumour is a group of cells that sustains proliferative signal, evades growth suppressors, resists cell death, induces angiogenesis and activates metastasis [4, 5].

The change in gene expression without any alteration in nucleotide sequence is termed as epigenetics [6, 7]. Epigenetic silencing of tumour suppressor gene and activation of oncogene is mediated by aberrant CpG methylation, histone modification and alteration in DNA binding protein [8].

DNA methylation is a epigenetic mechanism where modification of the genome is by covalent addition of a methyl group to DNA, mainly at cytosine residues within CpG islands of promoter region of human genes [9, 10]. In almost all cancer global hypomethylation and promoter hypermethylation is found. Hypomethylation of the genome causes chromosomal instability and elevation in mutation rates [11, 12]. Promoter hypermethylation causes point mutation and inactivation of tumour suppressor gene but global hypomethylation leads to activation of oncogene [13, 14]. There are biochemical [15, 16, 17], molecular [18] and nanotechniques [19, 20, 21] for DNA methylation detection. Each technique is previously reviewed in detail [14].

In this study, genome wide methylation analysis was carried out in blood of volunteers which could facilitate the development of accurate biomarkers for detection, diagnosis, prediction of response to therapy and prognosis of outcomes.

EXPERIMENTAL SECTION

In this study, genomic DNA was isolated from blood samples of 55 healthy volunteers (unknown cancer occurrence) were obtained after getting their concern and global DNA methylation was analysed to predict the risk of cancer in our locality.

Blood samples from people with a known family history of cancer were obtained and genomic DNA was isolated by standard phenol –chloroform extraction. The quality and quantity of DNA was checked using spectrophotometer. Methylation quantification was done using Epigenetek Methylation quantification Kit(Catalog No:P-1034)

In this assay, DNA is bound to strip wells that are specifically treated to have a high DNA affinity. The methylated fraction of DNA is detected using capture and detection antibodies and then quantified calorimetrically by reading the absorbance in a microplate spectrophotometer. Amount of methylated DNA is proportional to the Optical Density (OD) measured in microplate reader.

Chemicals required and Detailed experiment

The Chemicals phenol/chloroform/isoamyl alcohol proteinase K, Tris HCl, EDTA, sodium acetate, argrose, Ethidum bromide were obtained from Himedia The absorption spectra of DNA were measured using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The Agrose gel was documented using BioRAD1 Gel Doc System. The amount of methylated DNA was analysed using ELISA reader.

Blood collection

Blood Samples were also collect from the same volunteers by finger-pricking using sterile lancets and samples were kept in an EDTA-rinsed micro centrifuge tube. The tubes are stored at -20 °C for DNA extraction

Genomic DNA sample preparation

The genomic DNA from 55 healthy volunteers was isolated and visualized on an ethidium bromide stained 1% agarose gel and its was (Figure). The high molecular genomic DNA fragments were confirmed by 5000kb Ladder. The quality and quantity of the isolated DNA was determined with a spectrophotometer. After that the OD values at 260 nm were taken for estimating the DNA concentration. The OD 260/280 ratio was calculated for checking the purity of DNA;then DNA was stored at -20°C for further analysis.

Methylation Quantification

In each measurement, 100 ng of extracted DNA was taken for methylation analysis. The strip well was washed followed by detection antibody and enhancer solution was added and then DNA was bound to assay well. Again well was washed and capture antibody was added and change of colour from blue to yellow was observed .Intensity of colour is measured using ELISA reader. The relative methylation percentage status of two different DNA samples was determined using the following formula:

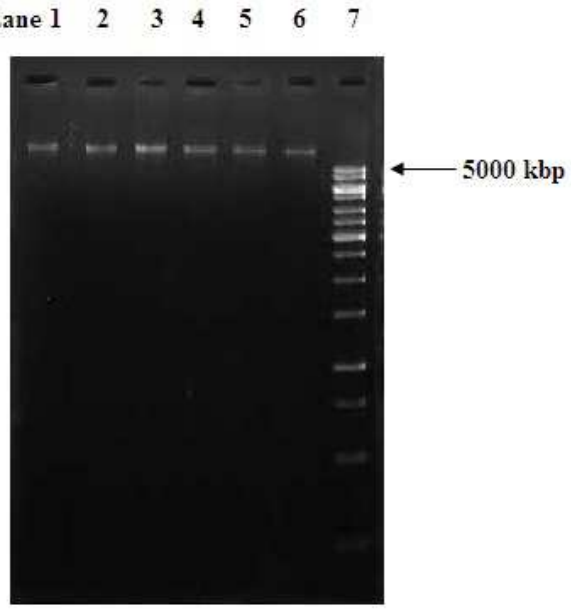
$$5\text{-mC \%} = \frac{(\text{Sample OD} - \text{ME3 OD}) \div S}{(\text{ME4 OD} - \text{ME3 OD}) \times 2^{*} \div P} \times 100\%$$

Where, S is the amount of input sample DNA in ng. P is the amount of input positive control (ME4) in ng. * 2 is a factor to normalize 5-mC in the positive control to 100%, as the positive control contains only 50% of 5-mC.

Results and Discussion

DNA was extracted and figure 1 shows the gel containing the bands of genomic DNA. The concentration of the DNA was also checked and it was found to be varying from 0.1 µg/µl to 0.7 µg/µl. The study participants were grouped based on their age, smoking, drinking, dietary habits and cancer history. The methylation frequency was calculated between Smokers/Non Smokers, alcohol users/non users, Vegetarian/Non Vegetarian, with/without cancer history and in different age groups.

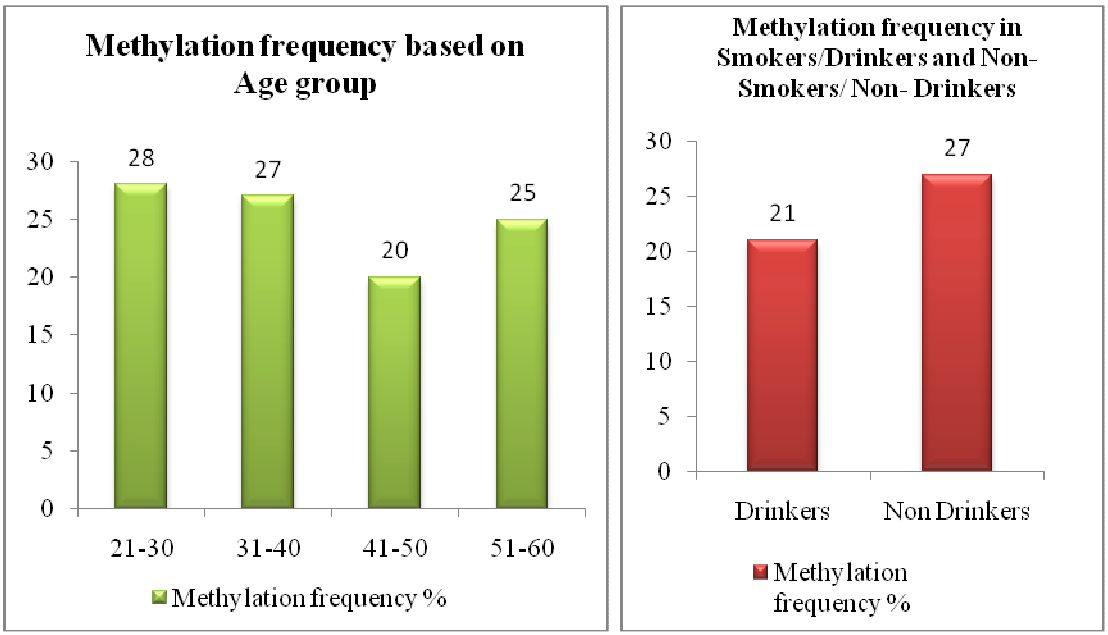
Figure 1. Agarose gel showing isolated Genomic DNA from Blood

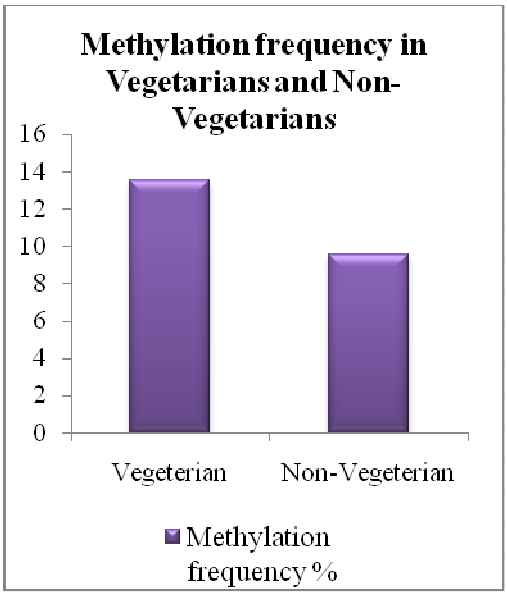


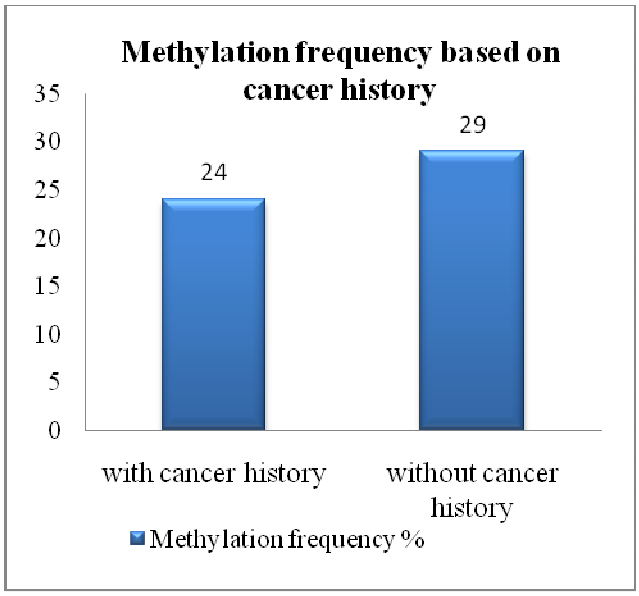
Lane 1 -6 : Genomic DNA greater than 5000kb
Lane 7: 5000kb Ladder.

From figure 2, it is evident that, the percentage of methylation for smokers and alcohol intakers was 21% as compared to 27% in non smokers/non alcohol intakers. This result confirms that smoking and consumption of alcohol can trigger the mechanism of hypomethylation which affects chromosomal stability and gene expression. In the previous study, it has been reported that exposures to smoking and perfluoroalkyl compounds (PFCs) are associated with global DNA hypomethylation [22].

Figure 2. Methylation Pattern in Relation to Age, Smoking/Drinking, Dietary Habits and Cancer History







In the present study, from figure 2, volunteers under 51 – 60 showed methylation frequency of 25% whereas it was 20% in the age group of 41 – 50 years. The methylation percentage in the age group of 31 – 40 years and 21 – 30 years was 27% and 28% respectively. It has been reported that aging in mammals is associated with alterations in the amount and patterns of DNA methylation in somatic cells [23]. Total genomic deoxymethylcytosine (dMC) has been found to generally decrease during aging in various organisms.[23] The global loss of genomic DNA methylation in both the aging process and tumourogenesis may partly explain the fact that age is one the risk factor for cancer development.

The dietary habits also affect overall hypomethylation frequency figure 2 shows that in Non – vegetarians the methylation frequency was found to be 9.5% whereas it was 13.5% in Vegetarians. Based on their cancer history the methylation frequency was found to be 24% whereas it was 29% in persons without cancer history. This result shows cancer history also plays a part in hypomethylation.

There are various methods in detecting DNA methylation. Atomic force spectroscopy is one of the promising method in detecting DNA methylation. Our group has been working on using AFM techniques in various biological applications such as enzyme substrate reaction [24]. We are working towards developing AFM as an efficient tool in detecting DNA methylation.

CONCLUSION

In this present study genome wide hypomethylation level was identified in the DNA. DNA extracted from blood carries not only tumour-specific changes in its sequence but also distinctive epigenetic marks, namely DNA methylation.. The relationship between the hypomethylation level and the other parameters like age, smoking, drinking, dietary habits and cancer history were also evaluated. These parameters have an impact on the genome wide hypomethylation level which could lead to the development of genomic instability cancer.

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