

Methylation profile of $p14^{ARF}$ / $p16^{INK4a}$ genes in gas station female attendants of Rio de Janeiro

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INTRODUCTION

The mixture of BTX (benzene, toluene and xylene), present in gasoline, is possibly carcinogenic. In Brazil, filling in fuel depends on attendants, who are chronically exposed to BTX. The exposure lasts 8 hours or more a day, turning the attendants DNA vulnerable to the epigenetic modifications. Methylation is a DNA modification, adding of a methyl group (CH_3) to cytosine. This modification has important regulatory effects on gene expression, especially involving the CpG islands in the promoter regions of many genes. The $p16^{INK4a}$ and $p14^{ARF}$ tumor suppressor genes are encoded by the *CDKN2A* locus, which is mapped on chromosome 9p21, encoding cell cycle regulatory proteins in the p53 and RB pathways.

OBJECTIVE

Our aim is to detect hypermethylation pattern in the promoters of $p14^{ARF}$ / $p16^{INK4a}$ genes in blood from female attendants and to evaluate their epidemiological profile.

MATERIAL AND METHODS

Forty-two female attendants and 12 controls were included in this study. About 100 ng of genomic blood DNA was used for the amplification of the promoter region of $p14^{ARF}$ / $p16^{INK4a}$ genes. The method was MSP (Methylation Specific Polymerase) applying the EPITECT Kit, cat. 59104 (Qiagen, Hilden, Germany), according to manufacturer's instructions. Methylation-specific products were resolved in 10% polyacrylamide gels stained with silver. This study was approved by the Ethics Committee of HUPE, CAAE 34310014.9.0000.5259.

RESULTS

According to epidemiological analysis of this study, the average age was 32.3 years, and the average of menarche was 12.6 years. The exposure time of the gasoline was 2.5 years and the percentage of alcoholics and smokers was 47.6% and 19%, respectively. Among the 42 samples of blood DNA, 19% (8/42) were hypermethylated in the promoter of $p14^{ARF}$, and 74% (31/42) were hypermethylated in the promoter of $p16^{INK4a}$.

Table 1: Hypermethylation profile of $p14^{ARF}$ / $p16^{INK4a}$ genes

Promoter	hypermethylation
$p16^{INK4a}$	74% (31/42)
$p14^{ARF}$	19% (8/42)
$p14^{ARF}$ and $p16^{INK4a}$	7% (7/42)

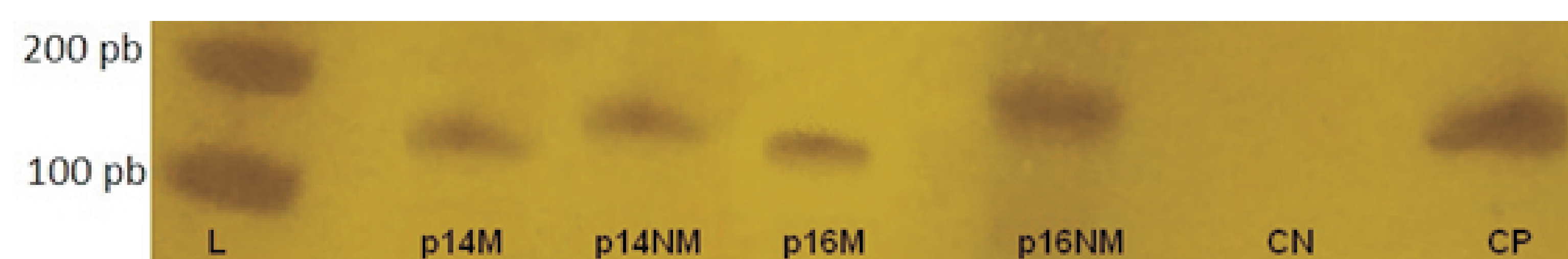


Figure 1: Methylation profile of the $p14^{ARF}$ / $p16^{INK4a}$ genes in gas station female attendants, in 10% polyacrylamide gels stained with silver. L, molecular weight marker; M, methylation; NM, unmethylation; CN, negative control (H_2O); PC, positive control (cell line DLD-1).

CONCLUSION

The promoter of $p16^{INK4a}$ was found more hypermethylated (3X) than promoter of $p14^{ARF}$. None of the 12 blood DNA samples were hypermethylated in the promoter of $p16^{INK4a}$. The analysis of the controls must be completed for conducting the statistical analysis. However, the partial results indicate that hypermethylation is more frequent in the female gas station attendants, indicating that these workers and their offspring have higher risk to cancer development.