EVALUATION OF THE INFLUENCE OF THE ADH3 ILE349VAL POLYMORPHISM IN THE FREQUENCY OF GENOTOXICITY BIOMARKERS IN WORKERS EXPOSED TO FORMALDEHYDE AND TOBACCO SMOKING

C LADEIRA 1,2, M.C. GOMES 3, M BRITO 1
1 Escola Superior de Tecnologia da Saúde de Lisboa – Instituto Politécnico de Lisboa
2 Centro de Investigação e Estudos em Saúde Pública – Universidade Nova de Lisboa
3 Faculdade de Ciências da Universidade de Lisboa

INTRODUCTION

Formaldehyde (FA) is a colourless gas widely used in the industry and hospitals as an aqueous solution, formalin. It is extremely reactive and induces various genotoxic effects in proliferating cultured mammalian cells.
Tobacco smoke has been epidemiologically associated to a higher risk of development of cancer, especially in the oral cavity, larynx and lungs, as these are places of direct contact with many carcinogenic tobacco's compounds. Genetic polymorphisms in enzymes involved in the metabolism are very important and can make changes in the individual susceptibility to disease. Alcohol dehydrogenase class 3 (ADH3), also known as formaldehyde dehydrogenase dependent of glutathione, is the major enzyme involved in the formaldehyde oxidation, especially in the buccal mucosa. The polymorphism in study is a substitution of an isoleucine for a valine in codon 349.
The cytokinesis-blocked micronucleus assay (CBMN) in human lymphocytes is one of the most commonly used methods for measuring DNA damage, namely the detection of micronucleus, nucleoplasmic bridges, and nuclear buds, classified as genotoxicity biomarkers.

AIM OF THE STUDY

Determine whether there is an *in vivo* association between genetic polymorphism of the gene ADH3 and the frequency of genotoxicity biomarkers in occupationally workers exposed to formaldehyde and with or without tobacco consumption.

METHODOLOGY

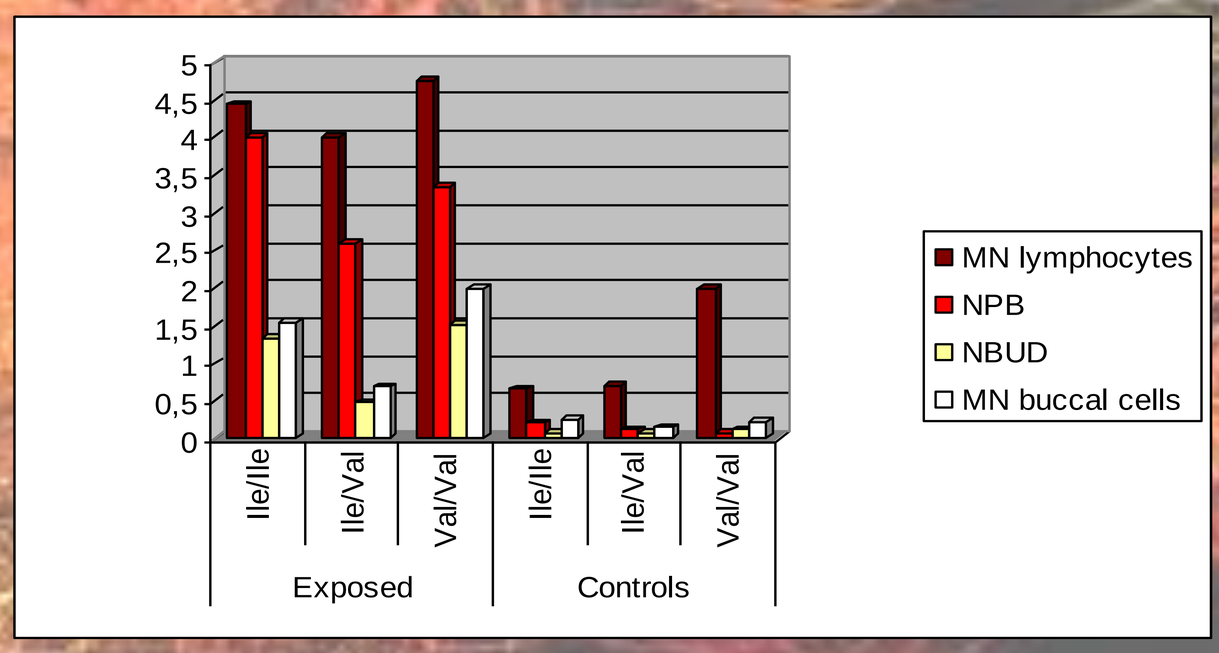
The study was carried out in Portugal in a sample of 56 workers occupationally exposed to FA in pathology anatomy laboratories and in 85 non-exposed subjects. Both groups were asked about their smoking habits among other items. The evaluation of genotoxic effects was conducted by applying CBMN in peripheral blood lymphocytes and the MN test in exfoliated cells from buccal mucosa. To perform the genotype analysis of ADH3 Ile349Val polymorphism, the target fragment was amplified using specific primers: *Forward* (5'-GCTTTAAGAGTAAATATTCTGTCCCC-3') e *Reverse* (5'-AATCTACCTCTTTCCSGAGC-3') and it was used PCR-RFLP. After, the digested products were analyzed in 4% agarose gel with ethide bromide. The data were analyzed statistically using Logistic Regression and Kruskal-Wallis test.

RESULTS

Table 1 – Descriptive statistics for genotoxcity biomarkers means according to ADH3 Ile349Val polymorphisms

Groups	ADH3	N	Mean MN lymphocytes ± S.E.	Mean NPB± S.E.	Mean NBUD± S.E.	Mean MN buccal cells ± S.E.
Exposed	Ile/Ile	15	4.44±0.974 (0-12)	4.00±0.811 (1-13)	1.33±0.361 (0-5)	1.53±0.710 (0-9)
	Ile/Val	34	4.00±0.723 (0-14)	2.59±0.722 (0-15)	0.47±0.185 (880-2)	0.68±0.301 (0-8)
	Val/Val	4	4.75±1.436 (3-9)	3.35±2.287 (0-10)	1.50±0.645 (0-2)	2.00±1.080 (0-5)
Controls	Ile/Ile	17	0.65±0.296 (0-4)	0.20±0.074 (0-2)	0.06±0.059 (0-1)	0.24±0.16 (0-2)
	Ile/Val	56	0.70±0.215 (0-7)	0.11±0.111 (0-3)	0.07±0.035 (0-1)	0.14±0.65 (0-2)
	Val/Val	9	2.00±0.645 (0-6)	0.06±0.059 (0-1)	0.11±0.111 (0-1)	0.22±0.222 (0-2)

Figure 1 – Distribution of the genotoxcity biomarkers according to ADH3 Ile349Val polymorphisms



CONCLUSIONS

The results concerning the lymphocytes suggest that MN in lymphocytes, NPB and NBUD are significantly associated with FA exposure (p< 0,001) but not with tobacco smoke. The results concerning the MN in buccal mucosa cells indicate that the FA exposure (p=0,012) and tobacco smoke (p=0,028) increase this biomarker but not in a synergist way. About the polymorphisms of ADH3, were found statistically significant, with the exception for NPB and MN in buccal cells. The polymorphism ADHE Ile/Val has a protective role in MN in lymphocytes and NBUD in comparison with Val/Val polymorphism. The analysis of the interaction between tobacco and FA exposure demonstrate that the absence of tobacco smoke in exposed to FA has a protective factor in NPB (p=0,000). There weren't observed any significant associations between tobacco consumption and FA exposure.
The development of biomarkers is one avenue to improved measurement of exposure, genetic susceptibility and disease. The majority of the single nucleotide polymorphisms in human genome are of low penetrance, including genes implicated in metabolism of chemicals. It is important to take into account that FA exerts its genotoxic action upon exposed human cells (both local and distant) and despite the potentially deleterious biological consequences outlined above, FA continues to be commercially utilized for disinfection, preservation and sterilization purposes.

REFERENCES

[1] – M. Pala, D. Ugolini, M. Ceppi, F. Rizzo, L. Maiorana, C. Bolognesi, T. Schilirò, G. Gilli, P. Bigatti, R. Bono, D. Vecchio, Occupational exposure to formaldehyde and biological monitoring of Research Institute workers, *Cancer Detection and Prevention 32* (2008) 121 – 126. [2] – S. Herausgegeben, U. Bernaurer, H. Mielke, U. Herbst, H.-B., Richter-Reichhelm, K.-E. Appel, U. Gundert-Remy, Assessment of the carcinogenicity of formaldehyde [CAS No. 50-00-0]. Berlim: Bundesinstitut fur Risikobewerfun – BFR, 2006. [3] – M. Berwick, P. Vineis, Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review, *Journal of the National Cancer Institute, 11* (2000) 874-897. [4] – L. Godderis, M. Boeck, V. Haufroid, M. Emmery, R. Mateuca, S. Gardinal, M. Kirsch-Volders, H. Veulemans, D. Lison, Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers, *Environmental and Molecular Mutagenesis 44* (2004) 293-303. [5] – L. Brooks, SNPs: Why do we care? Methods in Molecular Biology, vol. 212: Single Nucleotide Polymorphisms: Methods and Protocols. Edited by: P-Y. Kwok. Humana Press Inc., Totowa, NJ. [6] - S. Pavanello, E. Clonfero, Biological indicators of genotoxic risk and metabolic polymorphisms, *Mutation Research 463* (2000) 285-308. [7] – H. Cichoz-Lach, J. Partycka, I. Nesina, K. Celinski, M. Slomka, J. Wojcierowski, Genetic polymorphism of alcohol dehydrogenase 3 in alcohol liver cirrhosis and in alcohol chronic pancreatitis, *Alcohol & Alcoholism* (2005) 1-4. [8] – A. Groppi, J. Begueret, A. Iron, Improved methods for genotype determination of human alcohol dehydrogenase (ADH) at ADH2 and ADH3 loci by using polymerase chain reaction-directed mutagenesis, *Clinical Chemistry 10* (1990) 1785-1768. [9] – M. Osier, A. Pakstis, J. Kidd, J. Lee, S. Yin, H-C. Ko, H. Edenberg, R.B. Lu, K. Kidd, Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism, *Am. J. Hum. Genet. 64* (1999) 1147-1157. [10] – Hedberg, J. (2001). Function, expression and polymorphism of Human Alcohol Dehydrogenase 3/Glutathione-dependent formaldehyde dehydrogenase. Stockholm: Karolinska Institute. [11] – A. Yokoyama, T. Omori, Genetic polymorphism of alcohol and aldehyde dehydrogenases and risk for esophageal and head and neck cancers, *J Clin Oncol 33* (2003) 111-121. [12] - R. El-Zein, M. Schabath, C. Etzel, M. Lopez, J. Franklin, M. Spitz, Cytokinesis-Blocked Micronucleus Assay as a Novel Biomarker for Lung Cancer Risk, Cancer Res. 66:12 (2006) 6449 – 6456. [5] - R. El-Zein, M. Fenech, M. Lopez, M. Spitz, C. Etzel, Cytokinesis-Blocked Cytome Assay Biomarkes Identify Lung Cancer Cases amongst Smokers, Cancer Epidemiol. Biomarkers Prev 17 (2008) 1111 – 1119.