GENETIC POLYMORPHISM OF GLUTATHIONE S TRANSFERASE AND LUNG CANCER RISK IN NORTHERN ROMANIA

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Abstract

Background. *Polymorphisms for genes encoding glutathione S-transferase (GSTM1/GSTT1) might contribute to the variability in individual susceptibility to lung cancer. The role of GSTM1, and GSTT1 in lung carcinogenesis might be very important in exposure to carcinogens.*

Objectives. *This is a cross-sectional, randomized, case control study for the evaluation of the frequency of GST (GSTM1/GSTT1 null) alleles among patients with lung cancer.*

Subjects. *The study included 56 patients diagnosed with lung cancer. (histopathological examination), recruted from the Pneumology Hospital Leon Daniello Cluj and 125 healthy unrelated controls, selected among patients observed in the Internal Medicine Department.*

Methods. *GSTM1 and GSTT1 allele genotyping was carried out using Multiplex PCR amplification of relevant segment, followed by gel electrophoresis analysis.*

Results. *The molecular analysis identified GSTM null genotype in lung cancer patients was 53.9% compared to 47.6% of controls (φ=0.060, p=0.413). The prevalence of GSTT null genotype in lung cancer patients was 23.1% compared to 18.5% of controls. (φ=0.054, p=0.461).*

Conclusions. *The results of our study show no correlation between GSTM1 and GSTT1 null genotypes and lung cancer risk in Northern population of Romania.*

Keywords: Genetic polymorphism, Glutathione S transferase, Bronchopulmonary neoplasm.

POLIMORFISMELE GENETICE ALE GLUTATION S TRANSFERAZEI ŞI RISCUL DE A DEZVOLTA NEOPLASM BRONHOPULMONAR ÎN POPULAŢIA DIN NORDUL ROMÂNIEI

Rezumat

Premize. *Polimorfismele genetice implicate în metabolismul xenobioticelor par să fie un factor important în etiopatogenia neoplasmului bronhopulmonar. Glutation S transferazele au fost asociate cu susceptibilitatea crescută pentru anumite forme de cancer, inclusiv neoplasmul bronhopulmonar.*

Obiective. *Acest studiu randomizat, caz-control, are ca scop evaluarea frecvenţei alelelor nule GSTT1 şi GSTM1 la pacienţi diagnosticaţi cu neoplasm bronhopulmonar.*

Subiecţi. *Studiul include 65 de pacienţi cu neoplasm bronhopulmonar (diagnostic confirmat prin examen histopatologic), recrutaţi din cadrul secţiei Pneumologie I a Spitalului de Pneumoftiziologie Leon Daniello Cluj şi un lot martor de 125 de subiecţi fără pneumopatii sau neoplasme în antecedente, selectaţi din secţia*

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Metode. *Analiza polimorfismelor GSTT1 şi GSTM1 a fost precedată de amplificarea segmentului relevant (PCR Multiplex), fiind urmată de restricţia digestiei enzimatice pentru detectarea alelelor mutante.*

Rezultate. *Analiza moleculară a identificat alela nulă GSTM1 la 53.9% dintre pacienţii cu neoplasm bronhopulmonar, comparativ cu 47.6% dintre subiecţii lotului martor (φ=0.060, p=0.413). Prevalenţa alelei nule GSTT1 la pacienţii cu neoplasm bronhopulmonar a fost de 23.1%, comparativ cu 18.5% a lotului de control (φ=0.054, p=0.461).*

Concluzii. *Rezulatele preliminarii ale studiului nu au atins semnificaţie statistică pentru a stabili că prezenţa genotipurilor nule GSTT1 şi GSTM1 este asociată cu un risc crescut de a dezvolta neoplasm bronhopulmonar. Analiza frecvenţei genotipurilor nule, în raport cu tipul histologic tumoral, a evidenţiat o frecvenţă superioară a alelei nule GSTM1 la pacienţii cu adenocarcinom, comparativ cu celelalte tipuri histologice.*

Cuvinte cheie: polimorfism genetic, glutation S transferaza, neoplasm bronhopulmonar.

Aims

Lung cancer is a major cause of cancer-related death in Romania, with more than 10.000 new cases diagnosed each year. Although tobacco smoking is the primary cause of lung cancer, a genetic susceptibility factor also can modulate the risk of smoking-related lung cancer [1,2]. Everyone may have a unique combination of polymorphic traits that modify genetic susceptibility and response to drugs, chemicals and carcinogens. Developments in molecular biology have led to growing interest in the investigation of biological markers, which may increase the predisposition to lung carcinogenesis. Therefore, the high-risk genotype of an individual could be determined easily. As there are a great number of carcinogen-activating and -detoxifying enzymes, the variation in their expression and the complexity of exposures to tobacco carcinogens, the existence of multiple alleles at loci of those enzymes may result in differential susceptibilities of individuals. Establishing a correlation with the polymorphic alleles role in the metabolism of potentially carcinogenic toxic substances contained in cigarette smoke, and those that control inflammation and remodeling of lung tissue and cancer predisposition to develop lung has major clinical implications. Confirming a connection between these entities may have a high value in screening large populations by identifying individuals with genotypes with mutated alleles (heterozygous carriers, clinically healthy) who have increased risk of developing lung cancer [3].

Genetic polymorphisms reflect another approach to evaluating the role of genetic influences of lung cancer risk. Genetic polymorphisms are common variations in the genetic code, typically defined as comprising at least 1% of the population or sample of interest.

Manuscript received: 06.09.2011
Received in revised form: 12.09.2011
Accepted: 22.09.2011
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The results could provide new evidence and explanations that could answer the following question: ***"Why only some smokers*** (10% of the total number of those who smoke for more than 10 years) ***develop lung cancer?"*** [4].

The GSTs are a superfamily of genes whose products are phase II enzymes, catalyzing the conjugation of reactive intermediates to soluble glutathione [5]. GSTM1 is involved in degradation of active metabolites of polycyclic aromatic hydrocarbons (PAH) [6], whereas GSTT1 participates in detoxification of small hydrocarbons of tobacco smoke [7]. The lack of the detoxification activity is due to the inherited complete deletion of the respective genes [8,9]. Few important studies reported that generally the homozygous GSTM1 null genotype is more frequent than the GSTT1 null genotype [10,11]. Homozygous deletions of GST genes, alone or in combinations, have been associated with increased risk of lung cancer among Caucasians [12,13].

Patients and methods

The study was designed as a cross sectional randomized case control study. For this, a sample of randomly selected 56 cases with lung cancer were recruted from the cases admitted and followed in the Pneumology II Unit of Leon Daniello Pneumology Hospital Cluj-Napoca, Romania. Written informed consent was obtained from all subjects. Primary lung cancer was confirmed in all cases by pathological examination of a lung tissue sample. We must specify that all patients included in the study are active smokers (active smokers for more than 10 years). A sample of 2 ml of venous blood was then collected from all patients. Information Abstracted from the patients and medical records included pathology, family and personal history of lung pathology, lifestyle (tobacco, carcinogen exposure).

The total of 125 control subjects were recruited from the medical department of Medical Clinic II, Cluj

Emergency Clinical County Hospital. All patients were active smokers and have negative history for personal lung pathology (emphysema, lung cancer). After informed consent, blood samples were collected from all subjects, DNA Extraction and Purification was done using Wizard Genomic DNA Purification Kit (Promega).

Allele genotyping was carried out using multiplex PCR to determine the presence or absence of GSTT1 and GSTM1 genes. We used 3 pairs of primers to synthesize 215 bp (GSTM1 null genotype) and 480 bp (GSTT1 null genotype):

5'-GAACTCCCTGAAAAGCTAAAGC-3'; 5'- GTTGGGCTCAAATATAGGGTGG- 3'and

5'-TTCCTTACTGGTCCTCACATCTC-3'; 5'- TCACCGGATCATGGCCAGCA-3'

β Globin was co-amplified as an internal standard using the primer pair:

5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'

A total amount of 100 ng of genomic DNA was amplified in a total volume of 25 µl reaction mixture containing reaction buffer of 1.5 nM $MgCl_2$, 20 pmol of each primer, 200 µm of each dNTPs and 0.5 units of Taq polymerase.

Termocycling conditions were as carried out at 94 C for 5 minutes and then 35 cycles of 94 C for 1 min, 58 C for 1 min, 72 C for 1 min and a final polymerization step at 72 C for 10 min.

The amplification products were analyzed by gel electrophoresis (agarose 2%). The absence of amplification products was consistent with null genotypes. We must specify that this technique does not distinguish between heterozygous and homozygous positive individuals, but it identifies conclusively the null genotypes.

Statistical analysis was assessed by means of odds ration (OR) with 95% confidence limits calculated by logistic regression. GSTM1 and GSTT1 genotypes were classified as either null (homozygous deletion) or non-deleted. P value was also calculated for a more accurate risk evaluation.

Results

Sixty five (65) patients with lung cancer (12 women, 53 males, age mean 52,11), were included in the study. Control samples were obtained from 125 unrelated individuals (all smokers, > 65, no lung pathology) (43 females, 82 males, age mean 58.45).

The frequency of GSTM1 null allele in the study group was 53.9% comparing to 47.6% of the control group (φ=0.060, p=0.413). Also, the frequency of GSTT1 null allele in the study group was 23.1% comparing to 18.5% of the control group (φ=0.054, p=0.461).

Discussion and conclusions

In the present study, no statistical association between GSTT1 and GSTM1 and lung cancer etiology could be established. This result is in accordance with the limited data existing so far in the literature. A Review and Meta-Analysis regarding Glutathione S transferase and lung cancer [14], that involved 19,638 cases and 25,266 controls, observed a modest effect of the GSTM1 null variant on lung cancer risk, recent proof that the role of genetic polymorphisms of GST alleles may be overestimated.

Because of the complex pathways of carcinogen metabolism and the various enzymes involved, any single gene might play a smaller, more limited role in the risk of lung cancer. Larger, more comprehensive studies would allow to properly evaluating gene-environment in a complex disease such as lung cancer [14].

It also appears that the effect of the GSTM1 and GSTT1 genotypes may vary according to histological subtype. We evaluated the risk for each of three major lung cancer subtypes: squamous cell carcinoma (51 cases, 78.46%), small cell carcinoma (3 cases, 4.61%) and adenocarcinoma (16.92%). GSTM1 null variant was more frequent in patient with squamous carcinoma than GSTT1 null variant (54% compared to 21.5%). The same statistical result was found in adenocarcinoma patients, GSTM1 null allele was found in 54.54% of cases compared to GSTT1 null allele present in 18.18% of cases.

In conclusion, the results of our study show no correlation between GSTM1 and GSTT1 null genotypes and lung cancer risk in Northern population of Romania, although GSTM1 null variant seems to be associated with an increeased risk for lung adenocarcinoma.

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