Glutathione S-transferase T1 and myeloperoxidase −463 G>A genotypes in lung cancer patients of Kumaun region

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Abstract

Background: Null genetic polymorphism of Glutathione S-transferase T1 (GSTT1) and -463 G>A promoter polymorphism of myeloperoxidase (MPO) were studied for association with lung cancer. Materials and Methods: In a case-control study 26 lung cancer patients and 33 healthy individuals from hilly Kumaun region of northern India were investigated. DNA was extracted from peripheral blood. GSTT1 null polymorphism was detected by duplex PCR, and MPO polymorphism was detected by performing PCR-RFLP. Results: An increased but statistically non-significant risk for lung cancer was found for GSTT1 null genotype. No association for MPO -463G>A genotype was evident. Conclusion: Further study with large sample size may reveal such association in this population.

Key words: Glutathione S-transferase T1 null, kumaun region, lung cancer, myeloperoxidase −463G > A

INTRODUCTION

Lung cancer is the leading cause of cancer deaths world-wide. Active or passive smoking is considered as major cause of lung cancer. However, <20% of all smokers develop lung cancer. This suggests involvement of genetic variability in differential susceptibility for lung cancer in different individuals.

Genetic polymorphisms of enzymes involved in carcinogen metabolism may influence enzyme activity and thus lung cancer susceptibility. Glutathione-S transferase (GSTs) are phase II metabolic enzymes, which detoxify and excrete electrophilic metabolites including benzo[a]pyrene (B[a]P) and other polycyclic aromatic hydrocarbons found in tobacco smoke. GSTT1, a member of GSTθ class, contributes significantly in detoxification process. A null or total gene deletion that abolishes gene activity is present in population for GSTT1. This polymorphism has been associated in literature with lung cancer risk. Myeloperoxidase (MPO), a phase I metabolic enzyme, has been shown to convert metabolites of tobacco smoke procarcinogen (B[a]P) to highly reactive and carcinogenic intermediate benzo[a] pyrene diol epoxide that forms deoxyribonucleic acid (DNA) adducts. MPO has a single nucleotide polymorphism, -463 G>A at its promoter site that leads to loss of SP1 transcription factor binding site. Since this leads to reduced MPO expression, the variant allele A will lead to lesser production of carcinogenic intermediates. Thus it can be linked with reduced risk of lung cancer while the G allele with higher risk of the disease.

In an earlier study lung cancer was found as the leading cancer type in Kumaun region. Here we wanted to investigate association of GSTT1 null polymorphism and MPO promoter polymorphism with lung cancer in this region.

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MATERIALS AND METHODS

Study subjects
In total 59 individuals were studied. Out of them 26 were confirmed lung cancer cases. 33 normal healthy individuals were considered as controls. Lung cancer patients who were confirmed by tissue diagnosis and came for treatment at Swami Ram Cancer Hospital and Research Center, Haldwani, Uttarakhand were recruited as cases. Control group included unrelated healthy volunteers (blood donors) coming to Susheela Tiwari Government Hospital, Haldwani with no history of cancer. The study participants were interviewed for their locality, age, sex, individual as well as family health history and addiction habits. Signed informed consent was collected from each of them. Ethical approval for the study was obtained from Institutional Ethical Committee, Government Medical College, Haldwani.

Genotyping
5 ml of peripheral blood was collected in Sterile EDTA tube from each individual. DNA was extracted from whole blood following standard phenol-chloroform method.[12]

Detection of Glutathione S-transferase T1 (-/-)
Homzygous gene deletion polymorphism (-/-) for GSTT1 was detected by duplex (PCR). Primer set for GSTT1 genotype was lying within the GSTT1 gene[13] and another set of primer was selected from human mitochondrial manganese superoxide dismutase (MnSOD) as positive control of PCR [Table 1]. PCR reaction was performed in a 25 μl of reaction volume using 10 pmol of each forward and reverse primer (Eurofins, Bangalore), deoxyribonucleotide triphosphate (dNTP) mix (200 μM of each dNTP), and 0.5 U of Taq polymerase (Promega, USA) and PCR buffer provided along with the enzyme. The PCR condition followed an initial denaturation at 95°C for 1 min followed by 30 cycles of 30 s denaturation at 95°C, 30 s annealing at 58°C, 30 s extension at 72°C, and final extension at 72°C for 5 min. Negative control without template was set each time.

Table 1: Primer sequences and product size for PCR

| Gene   | Primer sequences                | Product size |
|--------|---------------------------------|--------------|
| GSTT1  | Forward 5' GGTCATTCTGAAGGCCAAGG 3'   | 131 bp       |
|        | Reverse 5' TTTGTGGACTGCTGAGGACG 3' |              |
| MnSOD  | Forward 5'AGCACCAAGGCAACGGCTGGCTCG 3' | 175 bp       |
|        | Reverse 5'CGGTGACGTTCAGGTTGTCACG 3' |              |
| MPO    | Forward 5'-GGTATAGGCACACAAATGGTGAG-3' | 350 bp       |
|        | Reverse 5'-GCAATGGGTTCAAGCGATTCTTTC-3' |            |

GSTT1: Glutathione S-transferase T1, MnSOD: Manganese superoxide dismutase, MPO: Myeloperoxidase, PCR: polymerase chain reaction

PCR products were checked in polyacrylamide gels stained with ethidium bromide under a gel documentation system. In duplex PCR absence of a 131 bp band indicated homozygous deletion (null; -/-) for GSTT1. Presence of this band indicated homozygous or heterozygous genotypes (+/+ or +/−, respectively). A 175 bp band of positive control MnSOD indicated successful PCR. This protocol did not differentiate between genotypes either with one or both copies of the gene. Duplex PCR were repeated for all samples showing null genotype.

Detection of –463G > A of MPO
PCR was carried out using 10 pmol of each forward and reverse primers [Table 1], PCR master mix (Promega) and PCR condition followed an initial denaturation at 94°C for 2 min followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 58°C, 30 s extension at 72°C, and final extension at 72°C for 5 min. Each set of reaction had negative control.

Single nucleotide polymorphism of MPO was detected by digesting 6 μl of PCR product with 0.5 U of Acil restriction enzyme (New England Biolab) at 37°C for 1½ h. Digestion product was visualized in polyacrylamide gel. Three bands at 169, 120 and 61 were produced for G/G homozygotes, two bands at 289 and 61 bp were produced for A/A homozygotes and heterozygotes (G/A) produced four bands at 289, 169, 120 and 61 bp. Randomly chosen five samples were sequenced (SciGenome, India) for confirmation of the restriction digestion results and the reproducibility was 100%.

RESULTS

The patient group was consisted of 24 males and 2 females. The control group included males only. Mean age of control group and cases were 28.68 ± 6.77 and 56.11 ± 9.22, respectively. Participants were mainly (95%) from hills, foothills and planes of Kumaun region. Rest of them (5%) was from adjacent hills and planes. Cases had markedly greater smoking history than controls (11.53% and 75.75% never smokers, respectively).

Number of individuals having different genotypes for GSTT1 and MPO are shown in Table 2. Incidences of GSTT1 null genotype was found to be higher in lung cancer cases (23%) than in control individuals (15%) although the difference was not statistically significant as found on the basis of odds ratio with 95% confidence interval value. For MPO polymorphism individual with AA genotype was not found in patients and only one was found in the control group. There was no difference in G allele frequency between cases and controls (0.86 in each group).
**DISCUSSION**

*GSTT1* is most ancient of all GSTs and found in almost all organisms investigated.\(^{[14]}\) Due to its role in carcinogen detoxification *GSTT1* has been studied for lung cancer risk in different environmental and ethnic backgrounds.\(^{[14]}\) However, different studies show contradictory results. Meta-analyses depict that there may be an association between increased lung cancer risk and *GSTT1* null polymorphism in Asians\(^{[14,15]}\) although no such association was evident in Caucasians.\(^{[15]}\) A weak association with lung cancer risk was observed in Turkish population.\(^{[9]}\) Further, smokers with *GSTT1* null genotype was found to have higher risk for lung cancer in a South Indian population.\(^{[6]}\) However, this genotype did not show any association with lung cancer risk in a Japanese population, or in another study on Turkish population.\(^{[16,17]}\)

Again, *MPO* promoter region polymorphism −463G > A has been linked with lung cancer. The variant allele was found to be protective from lung cancer risk in Caucasians.\(^{[7]}\) In a review Kiyohara et al.\(^{[19]}\) concluded that the variant form is linked with reduced risk of lung cancer in Turkish smokers also.\(^{[9]}\) The possible explanation for this protective effect is that the −463 AA/AG genotypes produce reduced level of smoking-related DNA - adducts.\(^{[19]}\) However, no association of this polymorphism for lung cancer was evident in other studies.\(^{[19,20]}\)

In our study an increased but statistically non-significant risk for lung cancer was observed for *GSTT1* null genotype. No association for *MPO* −463G > A genotype was found. The absence of significant association is probably due to small sample size. No elevated risk for lung cancer was observed for *GSTT1 - MPO* combined genotype also. However, to our knowledge this is the first report on these two polymorphisms and lung cancer risk in Kumaun region.

Smoking is an established etiological factor for lung cancer development. Strikingly in the present study all the smokers in patient group including one lady were bidi smokers. Only one patient was cigarette smoker. In India most common form of tobacco smoke is bidi. Since bidi imparts more risk for lung cancer than cigarette\(^{[21]}\) future investigations may include a comparative study on cigarette/bidi smoking and genetic association with lung cancer risk in this population.

**CONCLUSION**

*GSTT1* and *MPO* play important role in carcinogen detoxification process. Thus their variants those alter their normal function may be linked with lung cancer susceptibility. Although present data do not reveal any significant association, more extensive studies with larger sample size and considering other environmental factors, e.g. different carcinogenic sources may help to get better understanding of such genetic - variation and differential lung cancer susceptibility.

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**Table 2: GSTT1 and MPO genetic polymorphisms in relation to lung cancer in Kumaun region**

| Gene  | Polymorphism | Cases (%) | Controls (%) |
|-------|--------------|-----------|--------------|
| GSTT1 | Present (+/+ or +/−) | 20 (76.92) | 28 (84.84) |
|       | Null (+/−) | 6 (23.06) | 5 (15.15) |
| MPO   | G/G | 19 (73.07) | 25 (75.75) |
|       | G/A | 7 (26.92) | 7 (21.21) |
|       | A/A | 0 | 1 (3.03) |

**GSTT1:** Glutathione S-transferase T1, **MPO:** Myeloperoxidase
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