Abstract. Background: Cervical cancer is the leading cause of cancer-related death in women in developing countries. The -463 type G polymorphism of the MPO gene has been correlated with higher MPO expression and increased risk of various types of cancers. Our study was performed in order to evaluate the association between the -463G polymorphism and the prevalence of cervical cancer. Materials and Methods: In our prospective study, 149 patients with cervical cancer and 126 patients without any malignancies were enrolled. Results: No significant difference was found between genotype distributions in the cervical cancer patients and the control group. There was also no significant association of the genotype distributions with clinical prognostic factors. However, significantly lower A allele frequency of the -463 polymorphism of the MPO gene was found in our study group in comparison with that of the average Caucasian population. Conclusion: The -463 polymorphism of the MPO gene was not associated with susceptibility to cervical cancer.

Cervical cancer develops in the transformation zone of the cervix. Normal cervical cells may gradually undergo specific changes to become precancerous and then develop into cancer cells. Eighty to ninety percent of invasive cervical carcinomas are the squamous cell type which develop in flat, scaly surface cells that line the cervix.

Cervical cancer is the second most common cancer in women worldwide and is a leading cause of cancer-related death in women in developing countries (1). Moldavia has an incidence of cervical cancer ca. 15.1 cases per 100,000 women (2).

Infection with the human papilloma virus (HPV) has been suggested as a major risk factor for cervical cancer. Evidence of HPV is found in nearly 80% of cervical carcinomas (3) and a history of sexually transmitted disease has also been found to be associated with increased risk of developing cervical cancer (4). In addition, women who smoke cigarettes are twice as likely to develop cervical cancer (5). Various new predictive factors are under investigation to identify a high risk population for developing cervical cancer (6, 7).

Myeloperoxidase (MPO) is released from the cytoplasmic granules of neutrophils and monocytes by a degradation process. It reacts with hydrogen peroxide (H2O2) converted from the extra oxygen consumed in the respiratory burst to form a complex that can oxidize a large variety of substances. Among the latter is chloride, which is initially oxidized to hypochlorous acid, with the subsequent formation of chlorine and chloramines. These products of the MPO-H2O2-chloride system are powerful oxidants that have important roles in host defense by destroying a variety of targets including bacteria, fungi, viruses and non-malignant or malignant cells (8-15). However, the oxidant activity can also stimulate procarcinogens, cause H2O2-mediated DNA damage and compromise the repair process (16, 17). A polymorphic site is located 463 bp upstream of the MPO gene (-463G/A). The G allele acts as a strong SP1 transcription factor-binding site, which reacts with SP1 to increase MPO expression (18). The -463 type G polymorphism of the MPO gene, which is associated with a higher MPO expression level, has been correlated with an increased risk of various types of cancer, such as lung cancer (19-20), leukemia (21, 22), esophageal...
cancer (23), hepatoblastoma (24), bladder cancer (25) and ovarian cancer (26). However, contradictory results have also been observed (27, 28).

Lower or absent MPO activity has been observed in the peripheral blood neutrophils of women with cervical carcinomas, suggesting that lowered anti-tumor activity, operating mainly through the MPO-H$_2$O$_2$-halide system in these cells might contribute to the initiation of cervical cancer (29). Comparing the MPO level in the blood of cervical cancer patients with the -463 polymorphism genotype, different genotype carriers would be expected to have different susceptibilities for cervical cancer. Thus, the -463G/A polymorphism of MPO was analyzed in 149 cervical cancer patients and 126 healthy controls from Moldavia and the risk of developing cervical cancer by carrying the MPO polymorphism was estimated. In addition, the genotype distribution was also compared with the histopathological data of the cancer and age of the patients.

**Materials and Methods**

**Tumor samples and patients.** This case–control study was reviewed and approved by the Clinical Review Board and Ethics Committee of the Oncological Institute of Moldavia. Patients with histologically confirmed cervical cancer were included in this study. Written informed consent was provided by each patient. The control group consisted of similarly aged women with no history of cancer and all of them were treated for benign gynaecological diseases. None of them had previously undergone a hysterectomy. Only white Caucasian women were enrolled in this study.

EDTA-blood samples from 149 cervical carcinoma patients and 126 patients without any indication of any kind of cancer were collected in the Department of Gynaecology and Radio-Gynaecology of the Oncological Institute of Moldavia between 1999 and 2004. The histopathological diagnosis was confirmed by the Department of Pathology. The clinical staging was classified according to the criteria of the International Federation of Gynaecology and Obstetrics (30). The characteristics of the carcinomas and age of patients at diagnosis are shown in Table I.

**DNA extraction.** DNA was isolated from blood using commercial kits (DNA Extraction System I; ViennaLab, Vienna, Austria).

**Polymerase chain reaction (PCR).** The primer pair MPO-SE 5'-ATCTTGGGCTGTAGTGCC-3' and MPO-AS 5'-CCACATCATCAATTATTTCC-3' (GenBank accession no. M19507) was used to amplify a 238 bp fragment of the MPO DNA. The MPO-SE was biotinylated. The PCR was carried out in a total volume of 25 µl including 25 ng template, 5 pmol of each sense and antisense primer and puReTaq Ready-To-Go PCR Beads (Amersham Biosciences, England, UK), which contain 2.5 units of puReTaq DNA polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM MgCl$_2$, 200 µM dATP, dCTP, dGTP and dTTP, and stabilizers, including bovine serum albumin (BSA). The PCR was performed on a Perkin-Elmer GeneAmp PCR system 9700 with 40 cycles at 94°C for 30 sec, at 51°C for 30 sec and 72°C for 30 sec. The reaction was preceded by a primary denaturation step at 94°C for 1 minute and incubation at 72°C for 7 min.

**Results**

All samples were phenotyped by pyrosequencing, which demonstrated the phenotypic differences (Figure 1).

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Total</th>
<th>GG (%)</th>
<th>GA+AA (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>14</td>
<td>10 (71.4)</td>
<td>4 (28.6)</td>
<td>0.9257</td>
</tr>
<tr>
<td>Adenosquamous cell carcinoma</td>
<td>4</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>61</td>
<td>53 (86.9)</td>
<td>8 (13.1)</td>
<td></td>
</tr>
<tr>
<td>Keratinizing</td>
<td>69</td>
<td>46 (66.7)</td>
<td>23 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Non-keratinizing</td>
<td>6</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>26</td>
<td>18 (69.2)</td>
<td>8 (30.8)</td>
<td>0.6348</td>
</tr>
<tr>
<td>II</td>
<td>59</td>
<td>44 (74.6)</td>
<td>15 (25.4)</td>
<td></td>
</tr>
<tr>
<td>III+IV</td>
<td>61</td>
<td>48 (78.7)</td>
<td>13 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>3 (100.0)</td>
<td>0 (0.0)</td>
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</tr>
<tr>
<td>Grading</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>44 (75.4)</td>
<td>18 (24.7)</td>
<td>0.5478</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>9 (81.8)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>54 (78.3)</td>
<td>15 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7</td>
<td>6 (85.7)</td>
<td>1 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>89</td>
<td>72 (80.9)</td>
<td>17 (19.1)</td>
<td>0.0789</td>
</tr>
<tr>
<td>&gt;50</td>
<td>60</td>
<td>41 (68.3)</td>
<td>19 (31.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>113 (75.8)</td>
<td>36 (24.2)</td>
<td></td>
</tr>
</tbody>
</table>

GG, GA and AA: MPO gene polymorphisms.

Detection of polymorphisms by pyrosequencing. The G/A polymorphisms at -463 of the MPO gene were detected using a Pyrosequencer PSQ 96 and the PSQ 96 SNP Reagent Kit (Uppsala, Sweden (31)). A volume of 25 µl PCR product was used for pyrosequencing according to the instructions of the manufacturer. Five pmol of the sequencing primer MPO-SEQ 5'-CCTCAA GTGATCCACC-3' were applied to detect the polymorphism.

Statistical analysis. The Chi-square test was used to analyze the associations between genotypes and the histological type, differentiation grade and stage of cervical cancer, and the age of the patients dichotomized at 50 years. It was also used to compare the genotype distributions and allele frequencies between patients with cervical cancer and healthy women. In either population, violations of the Hardy-Weinberg-assumptions were statistically tested by comparing the observed genotype distribution with that expected under Hardy-Weinberg equilibrium again using a Chi-square test. In all analyses, the Chi-square test was replaced by Fisher’s exact test whenever an expected cell frequency was lower than five. The association of the risk of cervical carcinoma with the -463G/A MPO genotypes was expressed as an odds ratio estimate (OR) with a 95% confidence interval (CI). The statistical software package SAS V9.1 (2003 SAS Institute Inc., Cary, NC, USA) was used. A p-value of <0.05 was considered statistically significant.
There was no statistically significant difference between the genotype frequencies in the two groups (Table II).

The genotype distributions in each group were in Hardy-Weinberg equilibrium (Table II). The GG genotype frequencies were 75.8% and 70.6% in cervical cancer patients and in the control group, respectively. The difference was not statistically significant. The risk estimation showed a slightly increased risk by carrying the GG genotype. However, this was also statistically not significant (Table III).

Figure 1. Pyrogram of the pyrosequencing results. a: shows GA phenotype, b: shows AA phenotype, c: shows GG phenotype.
The statistical analysis also showed that there was no difference in the genotype distributions in cervical carcinoma patients with different histological types, tumor stage, histological grading, or age (Table I). There was also no statistically significant difference in the genotype distribution between patients aged 50 or younger and patients older than 50.

Discussion

There was no difference of the A allele frequencies in the cervical cancer patients and the cancer-free Moldavian women. However, the A allele frequency (0.15) was lower in comparison with most published data of the -463 polymorphism in MPO in Caucasian women (0.20-0.26) (18-20, 27-28, 31). Comparing our data with the study of Xu et al. (27) that investigated the highest number of cases (n=1128, 1784G+472A) to date, a statistically significant difference for the A allele frequencies between the Moldavian patients and controls, and other Caucasian populations (p=0.0003 and p=0.029, respectively) was found. Different genetic backgrounds in the Caucasian population have been reported (32). Stefan et al. identified the geographical region of Moldavia and the Carpathians as a break-point in the gene geography of Eastern Central Europe, providing a finer definition of one of the possible sharp genetic changes between Western and Eastern Europe (33). Our results are in concordance with this observation. Nevertheless, we have to acknowledge that this study was not designed as an epidemiological investigation. Therefore, more evidence is needed to reach a general conclusion based on this observation.

Despite the particular allele frequency of the Moldavian women, there was no difference in the genotype distribution between the cervical cancer patients and the control group, indicating that there is no difference in the risk of developing cervical cancer for different genotype carriers.

As described above, there have been contradictory results regarding the role of the MPO -463 polymorphism in the development of various types of cancer. Functionally, MPO has an initiating effect on tumorigenesis and a protective effect during tumor progression. The cervix is exposed to environmental infectious agents such as viruses and bacteria (34). The oxidant system is thus likely to be important for the effective control of “foreign” invasion. MPO might contribute to tumor initiation as it is known to cause DNA damage (15, 16). In contrast, it could also protect the host from cancer as it can assist in the elimination of malignant cells. Lower or absent MPO activity has been observed in the peripheral blood neutrophils of women with cervical carcinoma (29), indicating that women with a lower MPO activity might be susceptible to the development of cervical cancer. No correlation of carrying the GG genotype and a risk for cervical cancer was found, suggesting that either the MPO genotype is not directly correlated with MPO expression in the cervical tissue, or the MPO does not contribute to the initiation of cervical cancer. To answer these questions, the correlation of the MPO polymorphism with MPO activity and with patient outcome should be analyzed.

In conclusion, Moldavian women present a lower A allele frequency of the -463 polymorphism of the MPO gene than the average Caucasian population. Different genotypes of this polymorphism have no effect on the development of cervical cancer in Moldavian women.

References


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