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Assessment of myeloperoxidase (Mpo) gene polymorphism in cervical cancer

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Ethics Committee Approval
The ethical approval is obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee on 10.03.2011 with approval number 2011/07-14.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Cervical cancer (CC) is the most common gynecological malignancy in women. In spite of a variety of treatment protocols, it is necessary to carefully investigate all factors that play a role in the pathogenesis of these tumors which may have mortal progression. In this context, in our study we aimed to assess the myeloperoxidase (MPO) gene polymorphism, an important inflammatory enzyme, among cervix cancer cases.

Methods: In this cross-sectional study, 79 cases diagnosed as cervical carcinoma between 1992-2012 is included. The cases without archival paraffin blocks and clinical follow-ups are excluded. All slides with tumor involvement are reviewed and the ones which demonstrate tumor's characteristics are determined. After block determination 3 sections with 10-micron thickness obtained from paraffin blocks and MPO gene polymorphism was shown using acil restriction endonuclease enzyme with the restriction fragment length polymorphism (RFLP) method after polymerase chain reaction (PCR). The histopathological parameters including tumor stage and type, lymph node metastasis, ovary and endometrium involvement, recurrence and late metastasis are compared with genotype using chi-square and Fisher's exact test.

Results: The mean age of cases was 51.3 (10.9) years. Of 79 cases, 29 (36.7%) had AG (adenine-guanine) and 50 (633%) had GG (guanine-guanine) genotypes. Only endometrium involvement was identified to have a statistically difference with MPO gene polymorphism among the assessed histopathologic parameters (P=0.015). When clinical parameters are assessed, there was no difference identified between genotype and mortality (P=0.622).

Conclusion: Cervical cancer is thought to have progressive and regressive characteristics of tumor development due to the inflammatory response of the host. Within this framework, in our study assessing gene polymorphism of one of the inflammatory response foundation stones of MPO, we identified more endometrial involvement for cases with AG genotype. We believe this significance will be encountered for more parameters in broad case series.

Keywords: Cervical cancer, Myeloperoxidase, MPO, Gene polymorphism

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Introduction

Cervical cancer (CC) is the fourth common cancer in women according to 2018 cervical carcinoma incidences [3] and is accepted as the most common gynecological malignancy with more than 500,000 cases identified in 2012 [2]. The most common histologic type of this tumor, with death rates ranking 311,000 worldwide [1], is squamous cell carcinoma (SCC) comprising two thirds of cases [3]. The second most-common histologic type is adenocarcinoma. Both types of tumor have similar etiology. Like other epithelial tumors the tumor size, tumor invasion depth, lymphovascular invasion and lymph node and/or distant metastasis effects prognosis [4]. In cervical cancer, human papilloma virus (HPV) has an important role in both intraepithelial and invasive neoplasia [5]. For carcinogenesis associated with HPV, factors related to HPV in addition to the inflammatory response of the host to the virus play roles. It is considered that this inflammatory response may be responsible for both regression and progression of the lesion [6].

The human genome comprises approximately $3x10^9$ base pairs and nearly 50,000 genes carrying genetic information are contained within 46 chromosomes. Almost 99.9% of human DNA is identical between two people and the genetic variation (variability) among humans is sourced in small differences in the DNA chains. The differences in some DNA sequences do not affect human phenotypes but some directly leads to diseases. Between these two endpoints, there are genetic differences involving anatomy, physiology, treatment response, side effects of medication, tendency towards infection, and predisposition towards cancer [7].

DNA nucleotide changes in the form of more than one allele at a locus is called polymorphism. Alleles in more than 1% of chromosomes of the general population comprise "genetic polymorphism". If the incidence of alleles is less than 1%, than they are named as "rare variants". Polymorphic alleles found in the regulatory regions of genes affect transcriptional regulation of genes and may cause phenotypic changes [8].

Myeloperoxidase (MPO) is an enzyme that contains iron. It is found in the lysosome of monocytes, and granules of neutrophils. MPO uses hydrogen peroxide (H_2O_2) produced by neutrophils and produces hypochloric acid (HOCl) and other oxidants [9, 10]. HOCl may oxidize 10-20 times the amount of proteins that H_2O_2 does, and strong oxidant products produced by MPO may result in DNA injury. The only enzyme that is known to form HOCl is MPO. It inhibits tissue matrix metalloproteinase inhibitor 1 (TIMP-1) increasing matrix metalloproteinase activity and destroying proteins in the matrix. The MPO system is an important bactericidal. MPO expression is coded on a single gene with 14 kb length on the long arm of the 17th chromosome bounded by myeloid cells [11]. There is a polymorphic region in the MPO gene (-463G/A). On the MPO gene, -463 type G polymorphism is correlated with increased MPO expression and as a result increased risk of a variety of cancer types like lung, esophagus, bladder and ovarian tumors [12-15]. However, there are contradictory publications are also exist [16].

In cervical cancer cases, MPO activity has not been observed, or been observed to be low in peripheral blood neutrophils. It is considered that this reduced anti-tumor activity may play a role in development of cervical cancer [17]. As a result, in our study we aimed to research the correlation between MPO genetic polymorphism cases with prognostic histopathologic factors reported in the literature and survival.

Materials and methods

The ethical approval is obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee on 10.03.2011 with approval number 2011/07-14. After ethical approval, the study is conducted with 79 cases whom had "cervical carcinoma" diagnosis in Dokuz Eylul University Faculty of Medicine (DEUFM), Medical Pathology Department between 1992-2012. The cases without pathology archival material and clinical follow-ups are excluded from study. The non-pathological data are obtained from hospital information management system. Also the cases with no DNA extraction is not included in study.

The hematoxylin & eosin (H&E) stained slides with tumor involvement were reviewed and the slides which demonstrate tumor's characteristics is determined. The paraffin blocks that belongs to the selected slides are obtained from archive. 3 sections with 10-micron thickness are taken from these blocks and placed in 3 sterile and DNA-RNA free Eppendorf tubes. Between each case, the microtome device was cleaned with alcohol. Later DNA isolated from paraffinized sections had myeloperoxidase gene polymorphism determined with the PCR-RFLP method.

Genomic DNA paraffin commercial kits (Macherey-Nagel GmbH & Co. KG, Germany) were used. In the MPO gene (-463) polymorphic region, after polymerase chain reaction (PCR) (Biolabs Taq polymerase, M0320S, USA) restricted fragment length polymorphism (RFLP) was shown with Acil (Fermentas AciI, ER1791, Lithuania) restriction endonuclease enzyme.

PCR conditions to determine promotor region polymorphism of the MPO gene (-463):

1 cycle	94 °C for 5 min -preliminary denaturation
35 cycles	94 °C for 1 min – denaturation
	57 °C for 1 min – primer binding
	68 °C for 1 min - lengthening
1 cycle	68 °C for 10 min – final lengthening

Primer series;

MPO(F) 5′ - ACAGGTGAATCGCTGACATGCTGCCT - 3′
MPO(R) 5′- GAGACTCCCTGGAGGAAGAAGTTGAG - 3′

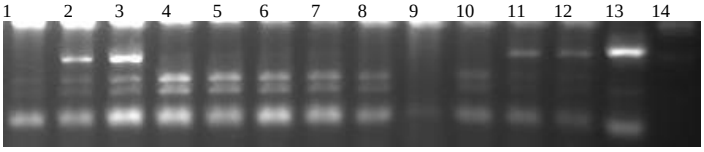
PCR product and fragment sizes;

PCR product;	350 base pair length
Fragment products;	AA genotype ; 289 + 61 base pair length
	AG genotype; 289 + 168 + 121 + 61 base pair length
	GG genotype; 168 + 121 + 61 base pair length

The obtained PCR products and products after enzyme fragmentation were separated in 2.5% agarose gel and stained with ethidium bromide to show in UV light. Figure 1 demonstrates a sample of DNA fragment analysis.

The PCR results are compared statistically with pathological parameters including tumor extensity, tumor type, lymph node involvement; parametrium, ovary, endometrium and abdominal fluid involvement.

Figure 1: Sample picture of DNA fragment analysis (Acil enzyme fragment) for MPO -463 promotor polymorphism stained with ethidium bromide (14: DNA length scale, 13: PCR product without fragmentation, 4-8,10: GG homozygote normal, 1-3,11,12: AG heterozygote mutant, 9: patient sample that could not be analyzed)



Statistical analysis

Statistical Package for the Social Sciences (SPSS) 15.0 program (SPSS Inc. Released 2006. SPSS for Windows, Version 15.0. Chicago, SPSS Inc.) is used for the analysis of the data. The Kolmogorov Smirnov test was applied to determine normal distribution of data for statistical analysis and then the chi-square test, Fisher's exact test, Kaplan Meier and regression analysis were used. Multivariate analysis could not be applied to create a model due to the lack of significance. And this cross sectional study results are reported according to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

Results

The study included 79 cases with ages varying from 27 to 72 years at time of diagnosis and mean age was 51.3 (10.9) years (median 52). Cases with DNA not obtained during PCR and with missing data were excluded from the study. Of the 79 cases analyzed, SCC was the most commonly observed tumor type (86.1%). The number of non-SCC tumors were low, therefore they were grouped for statistical analyses. The other histologic types apart from squamous cell carcinoma were adenocarcinoma (5.1%) and adenosquamous carcinoma (8.9%).

Of the 79 cases, 29 (36.7%) had AG (adenine-guanine) and 50 (63.3%) had GG (guanine-guanine) genotypes. Analysis with pathological parameters revealed 18 cases with tumor limited to the cervix had AG (32.7%) genotype while 37 had GG (67.3%) genotype. In cases which tumors were not limited to the cervix, these numbers were as 10 AG (47.6%) and 11 GG (52.4%). There was no statistically significant difference between tumor stage and genotype with the chi-square test (P=0.229). Cases with squamous cell carcinoma diagnosis showed 23 AG (33.8%) genotype and 45 GG (66.2%) genotype. Non-SCC tumors had 6 AG (54.6%) and 5 GG (45.5%) genotype. Similar to tumor extencity, statistical assessment with Fisher's exact test did not identify statistical significance between tumor type and genotype (P=0.199). Of the 65 cases (82.3%) without lymph node (LN) involvement, 23 had AG (35.4%) and 42 had GG (64.6%) genotype. For the 14 cases (17.7%) with lymph node involvement, 6 had AG (42.9%) and 8 had GG (57.1%) genotype. No statistical significance was detected in Fisher's exact test between LN involvement and genotype (P=0.761).

62 cases (78.5%) with parametrium (PM) involvement revealed 22 AG (35.5%) and 40 GG (64.5%) genotype. In the 17 cases (21.5%) without parametrium involvement, 7 showed AG (41.2%) genotype and 10 GG (58.8%) genotype. In Fisher's exact test no significant difference was detected between PM involvement and genotype (P=0.778). 75 cases (95.0%) without ovarian involvement were found to have 26 AG (34.7%) genotype and 49 GG (65.3%) genotype, while for 4 cases (17.7%) with ovarian involvement, 3 had AG (75%) and 1 had GG (25%) genotype. No statistical significant difference is observed in Fisher's exact test for ovarian involvement and genotype (P=0.137). Similar to these results, there was no statistical significance observed with Fisher's exact test for AG and GG genotypes with abdominal fluid involvement (P=0.059) and vaginal involvement (P=0.738). For the 68 cases (86.1%) without EM involvement, the number of cases with AG genotype was 21 (30.9%) and the number of cases with GG genotype was 47 (69.1%). For the 11 cases with endometrium (EM) involvement, 8 had AG (72.8%) and 3 had GG (23.2%) genotype. Fisher's exact test revealed a statistically significant difference between EM involvement and AG and GG genotypes (P=0.015).

75 cases (95.0%) were found to have at least 1 year disease free survival on follow-up, and among these cases 27 had AG (36.0%) and 48 had GG (64.0%) genotype. For 4 cases who were deceased, AG and GG genotypes were equal with 2 cases each (50%). No statistically significant difference is detected between genotype and death (P=0.622). Analysis of 77 cases with local recurrence & metastasis and genotype did not show statistically significant difference in Fisher's exact test (P=0.619, P=1.000, respectively).

Discussion

Cervical carcinoma was reported as the most common cancer in low resource countries in last decade [1]. In 2018, annually estimated new cases were 569,847 worldwide [18]. HPV is one of the most important factors for cervical carcinogenesis and this made the tumor preventable through vaccines. The increase in screening of cervix including non-invasive/ minimal invasive methods lead to detection of precancerous lesions and even tumor formation in early stage. Moreover the death rates are also decreased [1].

MPO is released by degradation of cytoplasmic granules in neutrophils and monocytes and is a strong oxidant material due to products of reactions with H_2O_2. These products have important roles in the defense of the host against harmful targets like bacteria, fungi, viruses, malignant or non-malignant cells [19, 20]. However, this oxidant activity may stimulate procarcinogens and result in DNA injury mediated by H_2O_2 [21, 22].

With the advances in molecular techniques in recent years, gene polymorphism and neoplastic processes have attracted attention as another research area. Gene polymorphism of the MPO enzyme, with an important place in inflammatory processes, is one of these entities [23, 24].

There is a polymorphic region on the MPO gene (-463 G/A). MPO gene -463 type G polymorphism causes increased MPO expression. This increased expression has different effects on a variety of tumor types in the literature. A study of pulmonary cancer histological types from 2002 identified the MPO -463 A allele was a marker of reduced risk of small cell carcinoma in the smoking population [25]. Similarly, a study including 91 esophagus tumor and 241 non-tumor cases, associated the MPO -463 A allele with reduced esophagus cancer risk [14]. Additionally, the MPO G-463A homozygote variant was reported to be associated with reduced bladder cancer [15].

Considering gynecological malignancies, a study including 125 ovarian tumor cases and 193 controls, did not identify a statistically significance between MPO and ovarian cancer risk [16]. In our study, data related to SC development were not present, but the difference between clinical data related to SC prognosis and MPO gene polymorphism was searched. There was no statistically significant difference between AG-GG genotypes and tumor stage, tumor type, LN status, metastasis presence, PM and ovarian involvement. However, analyses with Fisher's exact test identified a statistically significant difference between EM involvement and AG and GG genotypes. According to this data, it is shown that those with AG genotype had more EM involvement.

When studies assessing gene polymorphism in cervical carcinoma are considered, it is noted that p53 codon 72, CD40, CD83, IL10 and IL18 have been investigated [26-30]. In studies about MPO and cervix, it is reported that MPO gene polymorphism is not associated with cervical intraepithelial neoplasia (CIN) formation [31]. On a meta-analysis study consisted of 5 eligible studies mentioned that presence of polymorphism, −463 G > A might be protective for cervical cancer and they concluded that larger sample sized studies are needed in this area [32]. In our study we just had two genotypes which makes not possible to discuss the data. And it is worth noting that more studies needs be done to reveal the effect of MPO gene polymorphism on cervical carcinoma formation and prognosis.

Limitations

The number of cases included in the study is limited. Therefore the genotype homogenization is poorly achieved. We suggest that the statistical significance we identified for EM involvement will be encountered for more parameters in broad case series with more homogenized genotype distribution.

Conclusion

Our study though MPO gene polymorphism just detected relationship with EM involvement and AG genotype and we could not reveal association with the majority of histopathologic prognostic factors.

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