Association of xenobiotic-metabolizing genes polymorphisms with cervical cancer risk in the Tunisian population

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Title :

Association of xenobiotic-metabolizing genes polymorphisms with cervical cancer risk in the Tunisian population

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Abstract

Background: Host genetic characteristics and environmental factors interactions may play a crucial role in cervical carcinogenesis. We investigated the impact of functional genetic variants of four xenobiotic-metabolizing genes (*AhR*, *CYP1A1*, *GSTM1,* and *GSTT1*) on cervical cancer development in Tunisian women.

Methods: The *AhR* gene polymorphism was analyzed using the tetra-primer ARMS-PCR, whereas the *CYP1A1* polymorphism genotypes were identified by PCR-RFLP. A multiplex ligation-dependent polymerase chain reaction approach was applied for the analysis of *GSTM1* and *GSTT1* polymorphisms.

Results: The homozygous A/A genotype of the *AhR* gene (rs2066853) and the heterozygous T/C genotype of the *CYP1A1* SNP (*CYP1A1-MspI*) appeared to be associated with an increased risk of cervical tumorigenesis (OR^a = 2.81; OR^a = 5.52, respectively). Furthermore, a significantly increased risk of cervical cancer was associated with the *GSTT1* null genotype (OR^a = 2.65). However, the null *GSTM1* genotype showed any significant association with the risk of cervical cancer compared to the wild genotype (OR^a = 1.18; p = 0.784). Considering the combined effect, we noted a significantly higher association with cancer risk for individuals with at least two high-risk genotypes of CYP1A1/GSTT1 (OR^a = 4.2), individuals with at least two high-risk genotypes of CYP1A1/GSTT1/AhR (OR^a =

11.3) and individuals with at least two high-risk genotypes of CYP1A1/GSTM1/GSTT1/AhR exploitation low-risk genotype as a reference.

Conclusion: This study indicated that the single-gene contribution and the combined effect of xenobiotic-metabolizing gene polymorphisms (*AhR*, *CYP1A1-MspI*, *GSTM1,* and *GSTT1*) may have a considerable association with increased cervical cancer risk.

Keywords:

Cervical cancer**,** Xenobiotic-metabolizing genes, *AhR*, *CYP1A1*, *GSTM1,* and *GSTT1*.

Abbreviations:

SNP: Single Nucleotide Polymorphism

AhR: Aryl hydrocarbon Receptor

CYP1A1: Cytochrome P450 1A1

GSTM1: Glutathione S-transferase Mu 1

GSTT1: Glutathione S-transferase Theta 1

TAD: Transactivation Domain

TBP: TATA-binding Protein

WHO: World Health Organization

FIGO: International Federation of Gynecology and Obstetrics

SIL: Squamous Intraepithelial lesion

LSIL: Low grade Squamous Intraepithelial lesion

HSIL: High grade Squamous Intraepithelial lesion

ICC: Invasive Cervical Cancer

SCC: Squamous Cell Carcinoma

AC: Cervical Adenocarcinoma

HPV: Human Papillomavirus

PAH: Polycyclic aromatic hydrocarbon

Pap test: Papanicolaou test

PCR: Polymerase Chain Reaction

ARMS-PCR: Amplification Refractory Mutation System

PCR-RFLP: PCR-Restriction Fragment Length Polymorphism

SPSS: Statistical Package for the Social Sciences

OR^{a}: Adjusted Odd Ratio

1. Introduction

Cervical cancer is the fourth most common cause of cancer incidence and mortality in women worldwide [1]. Based on the World Health Organization (WHO) report in 2020, cervical cancer is ranked as the fifth most diagnosed cancer with an age-standardized incidence of 4.6/100000 in Tunisia [2]. This cancer slowly progresses from a precancerous lesion of intraepithelial squamous lesions (SIL), which divides into low-grade (LSIL) and high-grade (HSIL) stages, to invasive cervical cancer (ICC) [3]. Several studies have shown that human papillomavirus (HPV) may be the major risk factor for cervical cancer, which interacts with other risk factors, including lifestyle (smoking, oral contraceptive use, high parity, etc.), genetic predisposition, and environmental factors (air pollution, etc.). Air pollution is a major environmental and public health challenge worldwide, it contains a complex mixture of chemical elements that are known to be harmful to human health [4]. It is well known that dioxins and polycyclic aromatic hydrocarbons (PAHs) are among the most common pollutants in the world produced from incomplete combustion of organic materials and the manufacture of chlorinated hydrocarbons [5, 6]. Therefore, they may pollute the food chain and accumulate in fatty tissues and animal/human milk, given their liposolubility [7, 8]. Furthermore, PAHs were found particularly in urban areas, as characterized by polluted air from automobile emissions [4]. Dioxins and PAHs, which are mediated by the aryl hydrocarbon receptor (AhR), a ligand-activated transcriptional factor, play an important role in xenobiotic-induced carcinogenesis [9, 10]. AhR binds to a ligand and the formed complex was translocated into the nucleus and interacts with xenobiotic responsive elements. This induces the upregulation of phase I and II enzymes, such as cytochrome P450 1A1 (CYP1A1) and glutathione S-transferase (GST), which are involved in PAH metabolism [11]. Moreover, several reports have indicated that the functions of AhR may be influenced by the presence of genetic polymorphisms [12]. In this context, the c.G1661A single nucleotide polymorphism (SNP) (p.Arg554Lys; rs2066853) of the *AhR* gene was identified by Kawajiri *et al.*(1995) [13] as the most commonly studied polymorphism with malignancies including lung, breast, colorectal, and bladder cancers [14]. This functional genetic variant has an impact on the structure of the encoded protein by altering the affinity of the AhR receptors to toxic and carcinogenic ligands and affecting carcinogen metabolism [15]. In addition, *CYP1A1* and *GST* genes, encoding xenobiotic-metabolizing enzymes, contributed to carcinogen metabolism [16, 17]. The *CYP1A1-Msp*I variant (rs4646903), possessing T to C nucleotide substitution at the nucleotide 3801 in the 3'-flanking region of the gene, significantly affects the transcriptional efficiency and mRNA stability [18]. In numerous studies, the *CYP1A1-Msp*I polymorphism has been reported to be associated with the genetic susceptibility to cervical cancer. Moreover, several studies have reported that the lack of enzymes playing a role in the carcinogen's metabolism, such as GST, can increase the susceptibility to developing various types of cancer like cervical cancer [19, 20, 21, 22]. GST includes multi-isoform enzymes and the μ1 and Θ1 isoforms are encoded by the *GSTM1* and *GSTT1* genes respectively [23]. These genes are highly polymorphic leading to a wide variety in the expression and enzyme activity levels [24]. Indeed, a homozygous deletion in *GSTM1* and/or *GSTT1* genes, is frequently associated with the total loss of enzymatic activity of the respective proteins [25].

Altogether, these findings prompted us to explore the relationships between xenobiotic-metabolizing gene polymorphisms and the susceptibility of cervical cancer in Tunisian women. In this study, we investigated the distribution of single and combined SNPs of four relevant xenobiotic-metabolizing genes including *Ahr*, *CYP1A1*,

GSTM1, and *GSTT1* in a selected Tunisian population with cervical carcinoma. To our knowledge, this is the first report which explores the potential interaction between these genes in any pathology.

2. Materials and methods

2.1. Study population

This is a hospital-based case-control study including 100 healthy controls (mean age: 45 ± 11.34) and 71 patients diagnosed with cervical cancer (mean age: 45.26 ± 10.12) and collected from the Gynecology and Obstetrics Department at Tahar Sfar University Hospital, Mahdia, Tunisia, from April 2017 to May 2019. Inclusion criteria for all patients were 1) female sex; 2) age over 18 years; 3) no other malignancy and 4) no preoperative neoadjuvant therapy (chemotherapy or radiotherapy). Exclusion criteria were 1) age under 18 years; 2) have other chronic diseases; 3) have malignant tumors other than cervical cancer; 4) receiving radiotherapy or chemotherapy with unclear pathological diagnosis; 5) refused to participate. Screening and confirmation of cervical cancer diagnosis have been performed using bimanual pelvic and sterile speculum examination, Pap test, HPV typing test, colposcopy, and biopsy test. In our study, all confirmed patients have been a common diagnosis such as tumor location, tumor stage, histological type, and condition of lymph nodes. Cervical cancer groups consist of 58 cases of cervical squamous cell carcinoma (SCC), 7 cases of cervical adenocarcinoma (AC), and 6 cases of other pathological types of cancer. Tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org). Hence, 41 were staged I-II, 18 cases were staged III-IV and 12 cases were missing. Controls were selected by matching age and ethnicity with cases, during their routine gynecologic examination where they underwent a complete gynecologic examination including bimanual pelvic and sterile speculum examination, Pap test, and colposcopy. Moreover, controls do not have any type of cancer and diseases of the liver, kidneys, heart, brain, and vascular system as well gynecological inflammatory diseases. Information was obtained from participants through a standardized questionnaire including age, reproductive history, family history of cancer, and active exposure to smoke. This research was performed in accordance with the revised declaration of Helsinki [26]. All subjects in both groups provided informed consent to participate in the study and to allow their biological samples to be genetically analyzed. Approval of the study was given by the Bioethics and Research Committee of the Higher Institute of Biotechnology of Monastir (CER-SVS/ISBM 007/2021).

2.2. Genotyping

Total DNA was extracted from peripheral leukocytes using the salting-out procedure [27]. Four functional genetic variants were selected and analyzed by polymerase chain reaction (PCR), where all experiments were triplicated.

CYP1A1-MspI variant (rs4646903) analysis

The identification of the polymorphic variant *CYP1A1-Msp*I genotypes was performed using the PCR-restriction fragment length polymorphism (PCR-RFLP) method described by Bailey *et al.* [28]

A 340 pb PCR amplification fragment was generated using the primers 5'-CAG TGA AGA GGT GTA GCC GCT-3' (forward) and 5'-TAGGAGTCTTGTCTCATGCCT-3' (reverse)**.** Briefly, PCR was carried out in a total volume of 25 µl containing 0.5-1 µg genomic DNA, 1 µmol/l each of the forward and reverse primers, 1X Master Mix (2X Master Mix RED, Eppendorf, USA) with 1U of the Taq DNA Polymerase. Amplification conditions consisted of an initial denaturing step at 94 °C followed by 30 cycles of 95 °C for 30 s, 64 °C for 1 min, and 72 °C for 1 min. This was followed by a final extension step at 72 °C for 5 min. 5 µl of the PCR product was digested with *MspI* restriction enzyme (Thermo Fisher Scientific, USA) at 37 °C for 3 hours. Enzymatic digestion resulted in restriction fragments: the C allele was cut in two fragments of 200 and 140 pb while the T allele remained uncut [29].

***GSTM1* and *GSTT1* polymorphisms analysis**

Genotypes of *GSTM1* and *GSTT1* were analyzed by multiplex PCR as described by Babekir *et al.* [30]. The PCR amplification was carried out using the following primers:

GSTM1 variant: 5'-GAA CTC CCT GAA AAG CTA AAG C-3'; 5'-GTT GGG CTC AAA TAT ACG GTG G-3'.

GSTT1 variant: 5'-TTC CTT ACT GGT CCT CAC ATC TC-3'; 5'-TCA CCG GAT CAT GGC CAG CA-3'.

β-globin: 5'-CAA CTT CAT CCA CGT TCA CC-3'; 5'-GAA GAG CCA AGG ACA GGT AC-3'.

Briefly, PCR was carried out in a total volume of 25 µl containing 0.5-1 µg genomic DNA, 1 µmol/l each of the forward and reverse primers, 1X Master Mix (2X Master Mix RED, Eppendorf, USA) with 1U of the Taq DNA Polymerase. Amplification of the *β-globin* gene was used as an internal control. Amplification conditions consisted of an initial denaturation step at 95 °C for 5 min followed by 35 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, in the end, a final extension step at 72 °C for 5 min. For the analysis, 5µl of PCR product was fractionated on a 2% agarose gel. PCR products were directly visualized using a gel documentation instrument (Bio-Rad, USA).

The *GSTM1* and *GSTT1* genotypes were determined by the presence and absence (null) of the 215 pb and 480 pb bands, respectively, with internal control of 268 pb (beta-globin gene).

***AhR*- polymorphism (rs2066853) analysis**

Characterization of *AhR* genetic variant (rs2066853) was performed with the conventional tetra-primer amplification refractory mutation system PCR (ARMS-PCR). Four primers were designed for SNP analysis and synthesized by Macrogen, South Korea containing two outer (flanking) primers that served as an internal control of DNA integrity and two inner primers that target each specific allele (Table 1). The primers were designed using the PRIMER1 server [31]. PCR was performed in a 10 µl aliquot containing 1X Mastercycler gradient (2X Master Mix RED, Eppendorf, USA),1U of the Taq DNA Polymerase, and 1 µmol/l of each primer. Conventional PCR started with an initial denaturation at 96 °C for 5 min, followed by 35 cycles of denaturation step (96 °C, 30 s), annealing step (64 °C, 30 s), and extension step (72 C°, 50 s). The reaction was completed by a final extension of 72 °C for 5 min. Finally, all products were analyzed onto 2% agarose gel and visualized using the additive DNA Green Viewer™ and a gel documentation instrument (Bio-Rad, USA).

2.3. Statistical analysis

The data were examined using SPSS 21.0 software (SPSS Inc, USA). The adjusted odds ratio (OR^a) and its 95% confidence interval (95%CI) were calculated to estimate the association between variables and the risk of cervical cancer using unconditional logistic regression analysis with the low-risk genotype designated as the referent category. The effect of combined genotypes was analyzed between the study and control groups by unconditional logistic regression analysis with the absence of a high-risk genotype as the referent category. A *p-value* less than 0.05 was considered significant.

3. Results

The characteristics of women with and without cervical cancer are provided in Table 2. No significant differences in the distribution of marital status, age at menarche, menopausal status, number of childbirths, and contraceptive use were observed between cases and controls ($p > 0.05$). However, a significant decrease in cervical cancer risk was found among women who had a number of pregnancies > 5 ($p = 0.034$, $OR^a = 2.4$, 95% CI, 1.06-5.42) and did not have any family history of cancer ($p = 0.016$, $OR^a = 2.13$, 95% CI, 0.15-3.97). Table 3 shows genotype frequencies for *AhR* (rs2066853), *CYP1A1-MspI*, *GSTM1*, and *GSTT1* genes in patients and controls. They were in Hardy-Weinberg equilibrium. A significant difference in the *AhR* (rs2066853) SNP genotypes distribution was observed between cervical cancer cases and controls. Adjusted ORs for *AhR* (rs2066853) gene showed that the AA genotype had a 2.81-fold increased risk of developing cervical cancer compared to the GG genotype ($p = 0.026$). For the *CYP1A1-MspI* polymorphism, the frequency of TC genotype was particularly higher in patients than in controls (22.5% vs. 5%). Compared with the TT wild genotype, a significant positive association was found between the TC genotype and the risk of developing cervical cancer ($OR^a = 5.52$; $p = 0.002$). Moreover, the null *GSTT1* genotype was greater in patients (14.1%) than in controls (4%). Subjects carrying the null GSTT1 genotype exhibited more than a two-fold increase in the risk of developing cancer compared to those with the wild genotype ($OR^a = 2.65$; $p = 0.026$). In addition, the null *GSTM1* genotype showed any significant association with the risk of cervical cancer compared to the wild genotype ($OR^a = 1.18$; $p = 0.784$).

Given the functional interaction of the four studied genes (CYP1A1, GSTT1, GSTM1, and AhR) in the xenobiotic metabolism process, we investigated the combined effect of the simultaneous presence of this SNP on the development of cervical cancer. Tables 4 show the impact of having several high-risk SNPs for different combinations. In this context, the patients were classified according to the number of high-risk genotypes they carry based on our previous results. We identified high-risk genotypes: the TC genotype of CYP1A1, the AA genotype of AhR, the null genotype of GSTM1, and the null genotype of GSTT1. The low-risk genotypes will be used as the primary reference are the TT genotype of CYP1A1, the GG of AhR genotype, the wild genotype of GSTM1, and GSTT1. When we evaluated the combined effect of two gene polymorphisms, we remarkably noted that subjects with high-risk CYP1A1 and GSTT1 genotypes showed a higher estimated relative risk of cervical cancer with an adjusted OR of 4.2 ($p = 0.001$). Similarly, subjects with high-risk CYP1A1 and AhR genotypes showed a higher estimated relative risk of cervical cancer with an adjusted OR of 4.13 ($p = 0.001$). The study of the combined effect of three high-risk genotypes showed a significantly increased risk of cervical cancer among women carrying two

supposed high-risk genotypes of CYP1A1, GSTM1, and AhR (OR^a = 6.5, p = 0.023). The highest risk of developing cervical cancer was found among women carrying two high-risk genotypes of CYP1A1, GSTT1, and AhR using low-risk genotypes as a reference (OR^a = 11.3, p = 0.029). Similarly, the combined analysis of all studied gene polymorphisms showed a positive additive effect with cervical cancer. According to the data of this study, the most important combined effect of the studied genes was recorded in women carrying two high-risk genotypes. In this case, the risk of cervical cancer in these women was ten times higher (OR^a = 10.4, p = 0.003).

4. Discussion

Numerous factors are largely known to influence the incidence rate of cervical cancer combined with the highest amount of the HPV virus and smoking [32]. Moreover, low penetrance susceptibility genes are likely to be involved in cervical cancer risk. These minor genes do not directly cause carcinoma but, in concert with other genetic alterations or the interaction with certain environmental factors, may influence disease development [33, 34]. Considering these environmental factors, we count Dioxins and PAHs, which are a well-established group of chemical carcinogens [5, 35]. Interestingly, it is well known that the AhR which activates different xenobiotic-metabolizing enzymes such as CYP1A1 and the GST, mediates their cytotoxicity [10, 36]. The genes encoding *AhR* and these enzymes are genetically variable and functional polymorphisms affecting their coding regions or the regulation of their expression may contribute to inter-individual differences in susceptibility to and disease severity. The implication in carcinogenesis may be explored by analyzing the single effect of polymorphism or in combination with other polymorphisms. Thus, the risk of cancer incidence may potentially differ, according to these polymorphism distributions [37, 38]. In the present study, we investigated whether genetic variations in *AhR*, *CYP1A1*, *GSTM1*, and *GSTT1* genes might constitute risk markers for invasive cervical cancer in the Tunisian population. Furthermore, we performed a multilocus approach to analyze the combined effect of the four polymorphisms of the studied genes. To our knowledge, no previous epidemiological studies have dealt with the joint effect of these four main genes involved in the xenobiotic-metabolizing process. This study suggested a probable relationship between xenobiotic-metabolizing gene polymorphisms and cervical cancer incidence.

Concerning the human *AhR* gene, the most common polymorphism (rs2066853) was studied with several human malignancies including breast cancer, glioma, and acute leukemia which revealed a positive correlation between *AhR* polymorphism (rs2066853) and the susceptibility to cancer development [39, 40, 41]. In our study, we revealed that the A/A genotype of the (rs2066853) was the most frequent among women suffering from cervical cancer. The case-control results provided evidence of an association between the AA genotype and the risk of cervical cancer incidence compared to the G/G genotype (OR^a = 2.81; p = 0.026) indicating that the *AhR* polymorphism can serve as a candidate genetic screening marker. Similarly, several studies demonstrated a significantly increased risk of adult glioma and colorectal cancer with the A/A homozygous genotype (OR = 1.81, OR = 1.9; respectively) [40, 42]. Moreover, Chen *et al.* observed that the interaction between the A/A genotype of the *AhR* polymorphism (rs2066853) and cumulative cigarette smoking was significantly associated with an elevated risk of lung cancer in heavy smokers (OR = 3.36; p = 0.038) [43]. However, many other studies showed conflicting results reporting that the variant A allele for the (rs2066853) SNP showed a strong protective effect towards breast cancer and lung cancer (OR = 0.76, OR = 0.34; respectively) [44, 45]. On the other hand, previous data have demonstrated no statistically

significant association of *AhR* (rs2066853) polymorphism with cancer risk [37, 46]. A recent meta-analysis has reported that ethnicity and cancer type might be the source of the reported heterogeneity related to (rs2066853) polymorphism implication [12]. In the Tunisian population, there is a mosaic genetic structure, with the majority of the Eurasian lineages (Caucasians), followed by the Sub-Saharan and North African lineages. Accordingly, Gao *et al.* (2014) demonstrated that the *AhR* activity was higher in the variant allele A carriers of *AhR* (rs2066853) (G>A) polymorphism than in those carrying the GG genotype in a Caucasian population [47]. Consistent with previous data, our results confirm that genetically determined high activity of the A allele might be associated with increased cervical cancer risk. This *AhR* polymorphism may cause higher binding affinities, thus making their carriers more sensitive to environmental compounds exposure [48, 49]. Aftabi *et al.* (2016) reported that the *AhR*-G1661A transition may affect the local secondary structure and alter solvent accessibility, hydropathy features, post-translational modification, and pattern of the binding site residues in the acidic sub-domain of AhR-TAD (Transactivation domain). Consistently, these findings intensify the idea that this *AhR* transition affects TAD interactions, especially with the TBP (TATA-binding protein), which influences *AhR*-target genes expression [50]. Moreover, previous studies have observed that the *AhR* (rs2066853) variant displays an increased ability to induce the transcription of *CYP1A1* activity in lymphocytes compared with the common genotype after induction with 3-methylcholanthrene [51]. CYP1A1 is a key enzyme of the CYP1 family related to the metabolism of many endogenous substrates and environmental procarcinogens. It is well known that CYP1A1 activity may contribute to the formation of highly reactive intermediate metabolites, and these metabolites can form DNA adducts, which if obstructed, would initiate or promote oncogenesis [52, 53]. Hence, it is possibly feasible that polymorphisms of genes generating functional alterations in xenobiotic-metabolizing enzymes such as CYP 1A1 and GST may be vulnerable factors in cervical carcinogenesis [54]. Regarding *CYP1A1-Msp*I polymorphism, our results showed that women carrying T/C genotype were more likely to have cervical cancer. Indeed, adjusted ORs for the *CYP1A1-Msp*I variant showed that the TC genotype was associated with a 5.52-fold higher risk of cervical cancer incidence compared to T/T genotype indicating that the CYP1A1 SNP could serve as a candidate genetic screening marker. Our data is in agreement with several studies which have reported the implication of the *CYP1A1* polymorphism in the development of some cancers. In fact, TC and/or CC genotypes of *CYP1A1-MspI* polymorphism were found to be significantly associated with several malignancies such as cervical cancer [55, 56, 57]. Regarding *CYP1A1-MspI* polymorphism, a persistent association with elevated cervical cancer risk emerged in examined meta-analysis study [58]. Consistently, Juárez-Cedillo *et al.* (2007), found that Mexican women carrying the *CYP1A1-Msp*I T/C genotype showed a significantly elevated risk of cervical cancer rate compared to Mexican women possessing the T/T genotype (OR = 3.7) [59]. In addition, several other reports revealed that in the Indian population, individuals with TC genotype of the *CYP1A1-MspI* polymorphism had a significantly higher risk of cervical cancer (OR = 2,29; OR = 2.76) [54, 60]. Nevertheless, the homozygous *CYP1A1-MspI* CC genotype has been shown to be strongly associated with cervical cancer risk among German and Malay females [61, 62]. On the other hand, no significant association between the *CYP1A1-MspI* polymorphism and cervical cancer risk was observed by other reports [63, 64]. Tan *et al.* (2016) indicated that ethnicity and the small sample size can be major factors in determining whether the C/C genotype of *CYP1A1-MspI* affects the risk of cervical cancer. Indeed, they indicated that the low prevalence of the C allele and the seemingly naturally rare C/C genotype, especially within the Caucasian communities, might

be a factor that leads to the under-estimation of the homozygous *CYP1A1-MspI* CC genotype significance as a risk factor for cervical cancer [62]. Considering that the main part of the Tunisian population has a Caucasian background, it seems that our results were completely in accordance with studies exploring the implication of xenobiotic-metabolizing gene polymorphisms in cervical cancer predisposition in Caucasian ethnic groups [65]. Furthermore, the absence of a genetic association between the CC genotype and cervical cancer susceptibility could be explained by the low CC genotype frequencies in our population and the restricted sample size. In a larger cohort study, Matos *et al.* (2016) showed that individuals carrying the TC or CC genotype were at a higher risk for developing cervical cancer compared with those carrying the homozygous *TT* genotype (2.29-fold and 3.05-fold respectively) [66]. Moreover, previous reports have indicated that it causes the production of several harmful chemical products derived from PAHs, which could bind to DNA and cause DNA damage especially when coupled with a subpar or compromised GST enzymatic mechanism [67, 68]. It is well known that polymorphisms in GST, detoxifying enzymes, have a critical role in protecting DNA molecules from damage [69]. The mu and theta classes of *GST* isozymes (*GSTM1* and *GSTT1*, respectively) participate in the detoxification of carcinogenic intermediates of polycyclic aromatic hydrocarbons [70]. Several reports have indicated that the null genotype of *GSTM1* or/and *GSTT1* genes are frequently related to the development of cancers [71, 72]. In the current study, the null GSTM1 genotype was found in 6% of cervical cancer patients and 7% of controls. Hence, the adjusted ORs showed that women carrying the null GSTM1 genotype had a 1.18-fold higher risk of cervical cancer incidence ($p = 0.784$). In addition, the null GSTT1 genotype was found among 4% of cases and 14.1% of controls, and the relative adjusted OR was 2.65 among women with cervical cancer ($p = 0.026$). However, our data are also in agreement with previous reports. Joseph et al. (2006) reported that deletions of *GSTM1* and *GSTT1* were eminent among Indian cervical cancer patients (53.7% and 16.3%, respectively) compared with healthy individuals (32.7% and 9.7%, respectively) [54]. However, Nishino et al. (2008), found significant differences only in the genotype distributions of GSTM1 added to the ratio of the GSTM1 null genotype which was significantly higher in Japanese cervical cancer patients than in controls OR = 1.83 [63]. Nevertheless, in many other studies interested in other malignancies the distribution of the *GSTM1* and *GSTT1* null genotypes frequencies did not reach a significant difference compared to healthy controls. For example, Kalacas et *al.* (2019) revealed that *GSTM1* and *GSTT1* deletions could not be a risk factor for breast cancer development among Filipinos, but a higher risk may be detected while these genetic variants were combined with lifestyle or environmental factors such as passive smoking and alcohol consumption [73]. Additionally, a higher frequency of *GSTT1* and *GSTM1* deletions was identified among infected women with HPV 16. Indeed, it has been reported that women with polymorphism deletion in *GSTM1* and *GSTT1* genes may have an increased risk of cervical cancer progression. The relationship between vulnerability and HPV infection has further supported the importance of genetic polymorphisms [54]. As shown in many cases, the effect of a single gene may be modest compared to its effect when it interacts in synergy with several other genes. In addition, risk prediction may be limited if we focus just on the individual impact of each genetic variant. Thus, a multigenic approach that combines different variants involved in the same path may give us a clear impact on each genetic variant and improve predictive capacity [74]. In this context, it was important to study the combined effect of our four xenobiotic-metabolizing gene polymorphisms (*AhR*, *CYP1A1-MspI*, *GSTM1,* and *GSTT1*), since AhR interacts with the other xenobiotic-metabolizing enzymes, CYP1A1, GSTT1, and the GSTM1.

As shown in Table 4, the combined effect of two gene polymorphisms revealed that the higher estimated relative risks of cervical cancer have been reported in women carrying *CYP1A1*/*GSTT1* high-risk genotypes and in women carrying CYP1A1/AhR high-risk genotypes (OR^a = 4.2, OR^a = 4.13; respectively). Concerning results on the combined effect of three high-risk genotypes, there is a significant increase in cervical cancer risk in women carrying two high-risk genotypes of CYP1A1/GSTT1/AhR (OR^a = 11.3, p = 0.029). The combined analysis of *CYP1A1*, *GSTM1*, *GSTT1* and *AhR* showed a statistically significant combined effect on increased cervical tumor susceptibility (OR = 10.4; p = 0.003). Biologically, xenobiotic-metabolizing gene polymorphisms may interact with each other, or, be in linkage disequilibrium with several other genetic variants, thus producing many interaction effects. Consistently, many previous studies have reported an association between the interactive effects of xenobiotic-metabolizing gene polymorphisms and the development of numerous multifactorial diseases such as cancer [46, 75, 76].

5. Conclusion

In conclusion, our results indicate that xenobiotic-metabolizing gene polymorphisms may have a significant effect on cervical cancer susceptibility. The main limitation of this study is the small sample size. The study should therefore be viewed as hypothesis-generating and should be followed by larger prospective and multiethnic studies to confirm our findings. Despite this limitation, our results illustrate the potential of using this gene genotyping as a predictive risk tool that can be used in clinical practice when coping with this malignancy. Future studies of other functional genetic variants are also required to understand the role of the xenobiotic-metabolizing genes SNPs in determining the risk and progression of cervical tumors. The discovery of additional genetic factors that contribute to cervical carcinoma risk should provide opportunities for new approaches to the prevention, detection, and treatment of this common malignancy.

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Author contributions

Study design: A. H. and A. K., Patient enrollment and sample collection: A. H., N. B., and H. B., Experiments, data collection, and data analysis: A. H., N. B., S.S., F. D., M. D., Z. H., and A. K. Manuscript writing: A. H., S. S., M. D., and A. K., All authors revised the manuscript and approved its final version.

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