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Research Article

Susceptibility of PAX8, PBX2 and TERT gene variants in Cervical Cancer Patients

Ying Zhou[1], Kashif Bashir[2*], Ayesha Qamar[2], Maida Sulaiman[2] and Muhammad Umar[3]

[1]Department of Gynecology and Obstetrics, The Affiliated Hospital of Bei-hua University, Jilin City, Jilin Province,132000, China

[2]Department of Biological Sciences, Superior University, Lahore, 54000, Pakistan

[3]Department of Allied Health Sciences, Superior University, Lahore, 54000, Pakistan

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***Corresponding author:** Kashif Bashir, Department of Biological Sciences, Superior University, Lahore, 54000, Pakistan, Tel: +92-333-6876947, E-mail: kashifbashir70@gmail.com

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ABSTRACT

Objective and background: This research aims to explore the potential of single-nucleotide polymorphisms in the PAX8 rs10175462, PBX2 rs2856437 and TERT rs2853669 genes as biomarkers for cervical cancer by examining their association with the disease.

Methodology: The study involved 600 participants, comprising 300 healthy controls and 300 patients. DNA was extracted from blood followed by PCR.

Results: A significant correlation with an approximately two-fold elevated risk of developing cervical cancer is noted in the (TT) homozygous mutant of the rs10175462 SNP of the PAX8 gene (OR = 2.91; p = 0.0001). A highly significant correlation is observed in the heterozygote genotype (GT) of the same SNP (OR = 0.46; p = 0.0001). Significant correlation with high risk of disease by one-fold is noted in heterozygote genotype (GA) of rs2856437 of gene PBX2 (OR = 1.88; p = 0.0003), while an increase in risk by one-fold is noted in homozygous mutant (AA) (OR = 1.05; p = 0.81). The homozygous mutant genotype (GG) in rs2853669 shows a significant correlation (OR = 0.36; p = 0.0001), whereas in the heterozygote genotype (AG), the risk increases by two-fold and exhibits a significant correlation (OR = 2.57; p = 0.0001).

Keywords: PAX8, PBX2, TERT, Polymorphism, Cervical cancer

1. Introduction

Globally, cervical cancer is identified as the fourth leading cancer in terms of incidence among women. According to a study conducted in 2022, global statistics indicate that 660,000 women acquired a diagnosis of cervical cancer. However, the mortality rate was over 350,000 women due to the disease[1]. According to a study, almost 3,197 women die annually due to cervical cancer. However, 5,008 women received a diagnosis of cancer. Within Pakistan, Cervical cancer is the third leading malignancy. It is the second leading condition among females aged 15-44 years[2]. In almost all instances, infection caused by human papillomavirus (HPV) is the major cause of the cancer, with the high-risk Human Papillomavirus subtypes 16 and 18 identified as the

primary causative agents[3]. Interestingly, although an estimated 80% of females are likely to contract a Human papillomavirus infection in their lives, most of the infections are temporary and only a small fraction progresses to invasive cancer. Cervical cancer also shows a high heritability rate, suggesting the presence of genetically susceptible factors[4]. A single genetic locus, HLA, is strongly and consistently associated with a greater susceptibility to cervical cancer on chromosome 6[5]. A comprehensive cancer-wide study detected genomic alterations and identified overlapping genetic influences among multiple malignancies using data from the UK Biobank and GERA cohorts. It was conducted in collaboration with the UK Biobank [6], which revealed two particularly significant variants: The SNP rs10175462 is located on chromosome 2q14.1 of the PAX8 gene, while SNP rs2856437 is situated in the PBX2 gene on chromosome 6p21.32. Located at chromosomal location 2q14.4, the PAX8 gene produces a transcription factor classified under the paired box family, implicated in epithelial tissues, including the cervix. The rs10175462 variant is an intronic change in PAX8, indicating a newly identified potential risk region. PBX2 also function as a transcription factor responsible for cell differentiation, proliferation and apoptosis. The SNP rs2856437 within PBX2 has shown a correlation with increased susceptibility to invasive cervical cancer and intraepithelial neoplasia[5]. Knowing these genetic changes, such as rs2856437 in PBX2, clarifies the molecular mechanisms of cervical carcinogenesis and may facilitate the creation of targeted preventive measures. This study focuses on exploring the relationship between PBX2 rs2856437 and cervical cancer within a specific community to better understand host genetic susceptibility to HPV-related cancers. Typically, TERT expression is limited to germ cells and embryonic stem cells, but abnormal expression of TERT protein and mRNA has been detected in many cancers, including cervical cancer [6]. TERT is frequently overexpressed and reactivated in numerous malignancies and cancer cells. Several cancers in different populations are associated with the TERT promoter SNP rs2853669[8]. The TERT gene contains multiple SNPs in its promoter region linked to increased cancer risk. One such SNP, rs2853669 (T>C), is common and located upstream at the ATG promoter site at -245 bp. Mutations affecting TERT promoter activity can influence tumour survival and recurrence[9-11]. About 90% of human solid tumors show telomerase activity and indefinite replication, likely due to increased expression of telomerase in both fibroblasts and normal epithelial cells, indicating its role in malignant transformation[12]. The main goals are to examine the potential links between these gene variations and cervical cancer and to investigate their relationship with various demographic factors in both patient and control groups. This research involves a genetic association analysis of these three polymorphisms to determine their involvement in a significant cervical cancer case-control series.

2. Materials and Methods

There were 600 individuals in the trial. 300 were healthy controls and 300 of whom were patients who were compared based on age, smoking habits and family medical history. The patients with cervical cancer were chosen from Anmol Hospital in Lahore, Jinnah Hospital in Karachi, Allied Hospital in Faisalabad and Pinum Hospital in Faisalabad. Control samples were obtained from hospital patients who frequently visited for examination. During the recruitment period, each patient filled out and signed a consent form. Each patient was interviewed to learn further about their demographics and history of tobacco use. Parallel to family history, their smoking status was also mentioned. Blood samples, each measuring 5mL, were collected from the participants (patients and controls) and transferred into 5 mL EDTA-containing vacutainers. The collected blood samples were transported to the laboratory of Superior University, Lahore. The samples were preserved at 4 °C in refrigeration until further research procedures were carried out. A DNA extraction kit from BJ Micro Lab was applied to extract DNA from whole blood and the process was followed with a few minor adjustments. The isolated DNA was measured using a NanoDrop and 1 1% agarose gel, which was then kept at -20°C. The Primer3 software was utilized to design primers specific to SNPs of genes for gene polymorphism examination. DNA was synthesized using PCR. Primers specific to the polymorphisms of the selected genes were designed using Primer3 software. The sequence of all primers is given in the supplementary **(Table 1)**. Following primer design, DNA amplification was performed according to the standard protocol described by Bashir, et al.[12], with minor modifications. PCR conditions were optimized individually for each primer set by adjusting primer concentrations, $MgCl_2$ concentration and annealing temperatures. After optimization, the PCR cycling conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at the optimized temperature for 1 minute and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 10 minutes, followed by a hold at 4°C. The amplified products were verified by electrophoresis on a 2% agarose gel. This protocol involved a single-step PCR amplification. The presence or absence of amplification was determined by visual inspection of the agarose gel electrophoresis results. The sequence of all primers is given in the **(Supplementary Table 1)**.

Table 1: Demographic table for Controls and Cases.

Variables	Case (n=300)	Controls (n=300)	Adjusted Odds ratio (95% CI)	P-value
Age (Y) Mean ±SD				
<44, n (%)	158 (53%)	201 (67%)	0.54 (0.39 - 0.76)	0.0004
>44, n (%)	142 (47%)	99 (33%)		
Family history				
Yes, n (%)	105 (35%)	10 (4%)	5.6 (7.96-30.62)	0.0001
No, n (%)	195 (65%)	290 (96%)		
Smoking status				
Smokers	22 (7%)	53 (17%)	0.36 (0.21- 0.62)	0.0002
Non-Smokers	278 (93%)	247 (83%)		

OR, odds ratio; CI, confidence interval; OR, CI and p-value calculated by regression analysis.

2.1. Inclusion criteria

Blood sample eligibility criteria include those who have voluntarily agreed and were capable of providing written informed consent. All individuals with cervical cancer who have been medically and histopathologically proven, all females and people over 44 years old who are either asymptomatic or exhibiting symptoms. People who have a family background of cervical cancer or have been exposed to radiation fall into high-risk categories.

2.2. Exclusion criteria

The exclusion criteria for blood sampling include Persons younger than 18 years. This study did not include women who had previously been diagnosed with HIV infection, autoimmune disorders or other types of cancers. Furthermore, those who already had cancer treatment or who did not provide sufficient DNA samples for examination were not included.

2.3. Statistical analysis

Statistical representation of smoking status, their age and family medical history was performed with the aid of Mean and standard deviation. Both nonsmokers/smokers and Yes/No groups were selected to indicate the demographic variables of smoking status, as well as their family history.

For the genes PAX8 (rs101754628), PBX2 (rs2856437) and TERT (rs2853669), the genotypic frequencies of patients and controls, as well as mutation and allele frequencies, were determined using the Hardy–Weinberg law. For the odds ratio (OR) calculation, results were analyzed using a 95% confidence interval (CI) and statistical significance was assessed based on the corresponding p-value by using the online software MEDCALC®. The standard deviation and mean were computed using the Microsoft Excel function.

3. Results

Analysis of the single-nucleotide Polymorphism (SNP) in the PAX8, PBX2 and TERT genes: Polymerase chain reaction (PCR) was employed to investigate a single-nucleotide polymorphism (SNP) in the PAX8, PBX2 and TERT genes among healthy controls and patients.

Demographic characteristics of patients and controls for polymorphic analysis: A total of 300 cervical cancer patient samples were gathered. Demographic characteristics are shown in Table 1. The primary attributes of the group study are: Age distribution was comparable between groups, with an average of 44 years for each. In the case and control groups, 47% and 33% were members of the age group 44< and 53% and 67% were members of the age group 44>. A significant correlation was observed among the age groups (OR =0.54; p = 0.0004). Family history is a significant factor in both cases and healthy groups. In the current study, the observation was based on family history. An estimated 35% of patients had a family history, while 65% did not. Almost 4% of the healthy control group reported a family medical history of cervical cancer, while the remaining 96% had no such history. Statistical analysis shows that family history exhibits a significant correlation to a 15-fold increase in disease susceptibility (OR=15.6; p=0.0001). Smoking status is classified into two categories, e.g., smokers and Non-Smokers. Both groups were analysed based on smoking status. In cervical cancer patients, 7% of individuals were smokers and 93% of individuals did not have a smoking status. 17% of the control group participants were smokers, while the remaining 83% were non-smokers. The findings reveal a statistically significant correlation between smoking status and cervical cancer (OR=0.36; p=0.0002).

PAX8, PBX2 and TERT genes Association with Cervical Cancer Patients: PCR polymorphism examination of the PAX8 gene was performed. The genotype distribution of frequencies of SNP (rs10175462) of the PAX8 Gene is given in **(Table 2)**. The (GT) heterozygous genotype of rs10175462 shows a significant correlation with Cervical Cancer patients (OR=0.46; p=0.0001). A significantly two-fold elevated risk of disease is associated with the (TT) mutant genotype of rs10175462 (OR=2.91; p=0.0001). Whereas, a combination of heterozygous and mutant genotypes (GT, TT) exhibits a statistical correlation with higher disease susceptibility (OR=1.11; p=0.50). The PBX2 gene was analyzed for polymorphism using PCR methodology. The genotype distribution of the SNP (rs2856437) of the PBX2 Gene is given in Table. 2. The heterozygous (GA) genotype of rs2856437 is significantly correlated with cervical cancer risk and it elevates the one-fold likelihood of developing the disease (OR=1.88; p=0.0003). At the same time, the mutant (AA) rs28564372 genotype demonstrates a significant correlation with an elevated risk of disease (OR= 1.05; p= 0.81). The combined dominant model (GA, AA) shows a statistically significant correlation with an elevated risk of cervical cancer by one-fold (OR=1.80; p=0.0003). PCR polymorphism analysis of the TERT gene was performed. **(Table 2)** shows the frequency distribution by genotype of SNP (rs2853669) of the gene TERT. In the heterozygous genotype (AG) of rs2853669, a significant correlation is observed with Cervical Cancer patients, suggesting a two-fold elevation in disease risk (OR = 2.57; p = 0.0001). The (GG) Mutant of SNP rs2853669 demonstrates a significant correlation with the disease (OR= 0.36; p= 0.0001). Combined dominant model (AG, GG) by one-fold shows a significant correlation to increased susceptibility of disease (OR=1.46; p=0.0002).

Table 2: Allele and genotype frequencies of selected SNPs of PAX8, PBX2 and TERT in patients and controls.

SNPs	Genotypes	Case, n=300 (%)	Control, n=300 (%)	OR (95% CI)	p-value
rs10175462	GG	115 (38%)	123 (41%)	Ref (1)	
	GT	78 (26%)	129 (43%)	0.46 (0.33 - 0.65)	0.0001
	TT	107 (36%)	48 (16%)	2.91 (1.97-4.29)	0.0001
	GT+TT	185 (61%)	177 (59%)	1.11 (0.80-1.55)	0.50
rs2856437	GG	134 (45%)	178 (59%)	Ref (1)	
	GA	122 (41%)	80 (27%)	1.88 (1.33-2.65)	0.0003
	AA	44 (14%)	42 (14%)	1.05 (0.66-1.66)	0.81
	GA+AA	166 (55%)	122 (40%)	1.80 (1.30-2.49)	0.0003
rs2853669	AA	116 (39%)	133 (44%)	Ref (1)	
	AG	145 (48%)	80 (27%)	2.57 (1.82-3.62)	0.0001
	GG	39 (13%)	87 (29%)	0.36 (0.24-0.55)	0.0001
	AG+GG	184 (61%)	156 (17%)	1.46 (1.05-2.02)	0.0002

OR, odds ratio; CI, confidence interval; OR, CI and p-value calculated by regression analysis.

Correlation between PAX8 Gene Polymorphism and Family History: Analysis of the rs10175462 polymorphism in the PAX8 gene demonstrated variation in its association with cervical cancer depending on family history. Among patients with a positive family history, the heterozygous genotype (GT) showed a significant correlation with disease (OR=0.51; p=0.33), while the mutant genotype (TT) was linked to an approximately two-fold increase in cancer risk (OR=2.2; p=0.31). In contrast, among patients without a family history, the (GT) genotype also exhibited a significant correlation with the disease (OR=0.46; p=0.0002) and the TT genotype revealed a stronger and statistically significant correlation, conferring nearly a two-fold elevated risk of cervical cancer (OR=2.97; p=0.0001). These findings indicate that the TT genotype of rs10175462 represents a consistent risk factor for cervical cancer, with the association being more pronounced and statistically significant in patients lacking a family history of the disease. The rs2856437 polymorphism of the PBX2 gene exhibited differential associations with cervical cancer risk in family history. Among patients with a positive family history, the heterozygous genotype (GA) demonstrated an elevated risk of disease (OR = 1.61; p = 0.50), while the mutant genotype (AA) showed a non-significant association with increased susceptibility (OR = 1.50; p = 0.71). In patients without a family history, the (GA) genotype revealed a stronger and statistically significant correlation with higher cervical cancer risk (OR = 1.88; p = 0.001), whereas the (AA) genotype again showed a non-significant increase in risk (OR = 1.06; p = 0.82). Collectively, these findings suggest that the (GA) genotype of rs2856437 is a key contributor to cervical cancer susceptibility, with the effect being more pronounced and statistically robust in patients lacking a family history, while the (AA) genotype does not appear to exert a significant independent effect. The rs2853669 polymorphism of the TERT gene exhibited differential associations with cervical cancer risk in family history. Among patients with a positive family history, the heterozygous genotype (AG) demonstrated a significantly correlated two-fold elevated risk of disease (OR = 2.12; p = 0.29), while the mutant genotype (GG) showed a significant correlation with the disease (OR = 0.35; p = 0.17). In patients without a family history, the (AG) genotype revealed a stronger and statistically significant correlation with a two-fold higher cervical cancer risk (OR = 2.52; p = 0.0001), whereas the (GG) genotype showed a significant correlation with the disease (OR = 0.36; p = 0.0001). Collectively, these findings suggest that the (GA) genotype of rs2856437 is a key contributor to cervical cancer susceptibility, with the effect being more pronounced and statistically robust in patients lacking a family history, while the (AA) genotype does not appear to exert a significant independent effect. Association of PAX8, PBX2 and TERT polymorphisms with family history is given in **(Table 3)**.

Table 3: Association of PAX8, PBX2 and TERT polymorphisms with family history.

SNPs	Family history	Genotype	Patients n=300 (%)	Controls n=300 (%)	OR (95% CI)	p-value
rs10175462	Yes	overall	105 (35%)	10 (3%)		
		GG	40	4	0.92 (0.24- 3.47)	0.90
		GT	27	4	0.51 (0.13-1.98)	0.33
		TT	38	2	2.2 (0.45-11.23)	0.31
	No	overall	195 (65%)	290 (96%)		
		GG	74	119	0.87 (0.60-1.27)	0.49
		GT	51	125	0.46 (0.31-0.69)	0.0002
		TT	70	46	2.97 (1.93- 4.56)	0.0001
rs2856437	Yes	overall	105 (35%)	10 (3%)		
		GG	47	6	0.54 (0.14-2.02)	0.36
		GA	43	3	1.61 (0.39-6.61)	0.50
		AA	15	1	1.50 (0.17-12.71)	0.71
	No	overall	195 (65%)	290 (96%)		
		GG	87	172	0.55 (0.38-0.79)	0.0015
		GA	79	77	1.88 (1.27-2.77)	0.0013
		AA	29	41	1.06 (0.63-1.77)	0.82
rs2853669	Yes	overall	105 (35%)	10 (3%)		
		AA	41	4	0.96 (0.25- 3.61)	0.95
		AG	50	3	2.12 (0.52-8.65)	0.29
		GG	14	3	0.35 (0.08-1.55)	0.17
	No	overall	195 (65%)	290 (96%)		
		AA	76	128	0.80 (0.55- 1.16)	0.25
		AG	94	78	2.52 (1.72- 3.70)	0.0001
		GG	25	84	0.36 (0.22-0.58)	0.0001

OR, odds ratio; CI, confidence interval; OR, CI and p-value calculated by regression analysis.

Association of the PAX8 gene with AGE: Analysis of the rs10175462 polymorphism in the PAX8 gene revealed age-related differences in its association with cervical cancer risk. Among patients aged ≥44 years, the heterozygous genotype (GT) showed a non-significant association with disease susceptibility (OR = 0.47; p = 0.0008), while the mutant genotype (TT) exhibited a statistically significant two-fold elevation in risk (OR = 2.90; p = 0.0001). In contrast, among patients aged **≤44 years**, both the (GT) genotype (OR = 0.38; p = 0.0001) and the (TT) genotype (OR = 2.89; p = 0.0001) demonstrated significant associations

with cervical cancer, indicating stronger genetic susceptibility in this younger age group. These findings suggest that while the (TT) genotype consistently confers an increased risk across age groups, the GT showed a significant correlation with disease in individuals aged ≤44 years. The rs2856437 polymorphism of the PBX2 gene exhibited age-specific associations with cervical cancer susceptibility. Among patients aged ≥44 years, the heterozygous genotype (GA) showed a statistically significant correlation with the disease (OR = 0.54; p = 0.02), whereas the mutant genotype (AA) demonstrated a non-significant association with risk (OR = 1.05; p = 0.88). In patients aged ≤44 years, the GA genotype was significantly associated with an elevated risk of cervical cancer (OR = 1.85; p = 0.006), while the (AA) genotype again showed only a non-significant effect (OR = 1.05; p = 0.86). Collectively, these findings suggest that the (GA) genotype of rs2856437 confers a significant risk across both age groups, with the association being stronger in younger patients (≤44 years), whereas the (AA) genotype does not appear to exert a meaningful influence on disease susceptibility. The rs2853669 polymorphism of the TERT gene demonstrated a consistent association with cervical cancer risk across age groups. Among patients aged **≥44 years**, the heterozygous genotype (AG) was significantly associated with a two-fold elevation in disease risk (OR = 2.52; p = 0.001), while the mutant genotype (GG) also showed a significant correlation with cervical cancer (OR = 0.35; p = 0.001). Similarly, in patients aged **≤44 years**, the (AG) genotype conferred a significant two-fold increase in susceptibility (OR = 2.52; p = 0.0001) and the (GG) genotype again demonstrated a significant association with disease risk (OR = 0.37; p = 0.0005). These findings indicate that the (AG) genotype consistently elevates cervical cancer risk irrespective of age, while the (GG) genotype also shows a significant role, suggesting that TERT rs2853669 may act as a strong genetic determinant of cervical cancer susceptibility across different age groups. Association of PAX8, PBX2 and TERT polymorphisms with age is given in **(Table 4)**.

Table 4: Association of PAX8, PBX2 and TERT polymorphisms with age.

SNPs	Age years	Genotype / Alleles	Patients n=300 (%)	Controls n=300 (%)	OR (95% CI)	p-value
rs10175462	≤44	Overall	158 (53%)	201 (67%)		
		GG	61	83	0.89 (0.58- 1.36)	0.60
		GT	41	86	0.38 (0.24- 0.59)	0.0001
		TT	56	32	2.89 (1.76- 4.77)	0.0001
	≥44	Overall	142 (47%)	99 (33%)		
		GG	54	41	0.86 (0.51-1.46)	0.59
		GT	37	42	0.47 (0.27- 0.82)	0.008
		TT	51	16	2.90 (1.53- 5.48)	0.0001
rs2856437	≤44	Overall	158 (53%)	201 (67%)		
		GG	71	119	0.56 (0.36- 0.85)	0.007
		GA	64	54	1.90 (1.18- 2.89)	0.006
		AA	23	28	1.05 (0.58- 1.90)	0.86
	≥44	Overall	142 (47%)	99 (33%)		
		GG	63	59	0.58 (0.34- 0.97)	0.03
		GA	58	26	0.54 (0.32-0.90)	0.02
		AA	21	14	0.99 (0.50- 2.18)	0.88
rs2853669	≤44	Overall	158 (53%)	201 (67%)		
		AA	61	89	0.79 (0.51- 1.20)	0.27
		AG	76	54	2.52 (1.62- 3.92)	0.0001
		GG	21	58	0.37 (0.21- 0.65)	0.0005
	≥44	Overall	142 (53%)	99 (33%)		
		AA	55	43	1.93 (1.08- 3.77)	0.024
		AG	69	27	2.52 (1.45- 4.37)	0.001
		GG	18	29	0.38 (0.18- ٠,٦٧)	0.001

OR, odds ratio; CI, confidence interval; OR, CI and p-value calculated by regression analysis.

Association between PAX8 polymorphisms and smoking status: The rs10175462 polymorphism of the PAX8 gene demonstrated distinct associations with cervical cancer risk depending on smoking status. Among smokers, the heterozygous genotype (GT) exhibited a non-significant association with disease susceptibility (OR = 0.38; p = 0.09), whereas the mutant genotype (TT) was significantly correlated with a three-fold higher risk of cervical cancer (OR = 3.21; p = 0.04). In non-smokers, carriers of the (GT) genotype showed a significant association with increased disease risk (OR = 0.46; p = 0.0001), while the (TT) genotype was also significantly linked to a two-fold elevation in cervical cancer risk (OR = 2.86; p = 0.0001). These findings suggest that the (TT) genotype of rs10175462 consistently confers an increased susceptibility to cervical cancer, with the risk being more pronounced in smokers, whereas the (GT) genotype exerts a stronger and statistically significant influence among non-smokers. The rs2856437 polymorphism of the PBX2 gene showed varying associations with cervical cancer risk based on smoking status. Among smokers, the heterozygous genotype (GA) was associated with an elevated risk of cervical cancer (OR = 1.92; p = 0.21), though the correlation did not reach statistical significance, while the mutant genotype (AA)

displayed no meaningful association (OR = 0.88; p = 0.87). In non-smokers, the (GA) genotype demonstrated a stronger and statistically significant correlation with increased risk of cervical cancer (OR = 1.87; p = 0.0008), whereas the (AA) genotype again showed no significant association (OR = 0.94; p = 0.81). These findings indicate that the (GA) genotype of rs2856437 may contribute to cervical cancer susceptibility, particularly in non-smokers, while the (AA) genotype does not appear to play a significant role regardless of smoking status. The rs2853669 polymorphism of the TERT gene exhibited significant associations with cervical cancer risk to smoking status. Among smokers, the heterozygous genotype (AG) was correlated with a two-fold increase in disease risk (OR = 2.78; p = 0.05), while the mutant genotype (GG) also showed an association (OR = 0.36; p = 0.14), though the latter was not statistically significant. In non-smokers, the (AG) genotype demonstrated a strong and highly significant two-fold elevated risk of cervical cancer (OR = 2.55; p = 0.0001) and the (GG) genotype was similarly and significantly associated with disease susceptibility (OR = 0.36; p = 0.0001). These results suggest that the AG genotype consistently increases cervical cancer risk, while the (GG) genotype appears protective, with both associations being more robust and statistically significant in non-smokers. Association of PAX8, PBX2 and TERT polymorphisms with smoking is given in **(Table 5)**.

Table 5: Association of PAX8, PBX2 and TERT polymorphisms with smoking status.

SNPs	Smoking status	Genotype / Alleles	Patients n=300(%)	Controls n=300 (%)	OR (95% CI)	p-value
rs10175462	Smokers	Overall	22 (7%)	53 (17%)		
		GG	9	22	0.97 (0.35- 2.67)	0.96
		GT	5	23	0.38 (0.12- 1.19)	0.09
		TT	8	8	3.21 (1.01-10.14)	0.04
	Non-Smokers	Overall	278 (93%)	247 (82%)		
		GG	107	101	0.90 (0.63- 1.28)	0.57
		GT	72	106	0.46 (0.32-0.67)	0.0001
		TT	99	40	2.86 (1.88- 4.34)	0.0001
rs2856437	Smokers	Overall	22 (7%)	53 (17%)		
		GG	10	31	0.59 (0.21- 1.60)	0.30
		GA	9	14	1.92 (0.67-5.49)	0.21
		AA	3	8	0.88 (0.21-3.71)	0.87
	Non-Smokers	Overall	278 (93%)	247 (82%)		
		GG	124	147	0.54 (0.38- 0.77)	0.0007
		GA	113	66	1.87 (1.29-2.71)	0.0008
		AA	41	34	0.94 (0.58- 1.52)	0.81
rs2853669	Smokers	Overall	22 (7%)	53 (17%)		
		AA	8	24	0.69 (0.24- 1.92)	0.47
		AG	11	14	2.78 (0.98-7.84)	0.05
		GG	3	16	0.36 (0.09- 1.41)	0.14
	Non-Smokers	Overall	278 (93%)	247 (82%)		
		AA	108	109	0.80 (0.56- 1.13)	0.22
		AG	134	66	2.55 (1.76- 3.68)	0.0001
		GG	36	72	0.36 (0.23-0.56)	0.0001

OR, odds ratio; CI, confidence interval; OR, CI and p-value calculated by regression analysis.

4. Discussion

Cervical cancer ranks as the leading gynecological malignancy and is a serious global health concern and a large contributor to years of life with a disability[13]. However, only a limited number of hereditary risk factors have been identified to date. A malignant growth called cervical cancer develops in the cervix, the narrow passageway to the lower part of the uterus that connects to the vagina at the bottom. Our study seeks to provide a current overview of cervical cancer trends in Pakistan, focusing on incidence and mortality rates, while also updating the known risk factors and etiological contributors. It remains the third most prevalent cancer affecting women in Pakistan. Persistent infection with oncogenic strains of high-risk Human Papillomavirus is the principal ecological factor for cervical cancer. Chronic HPV infection, such as HPV16, HPV18, HPV31, HPV33, HPV35, HPV45, HPV52 and HPV58, plays a key role in initiating cervical dysplasia that may advance to cervical cancer, recognizing that HPV infection by itself is insufficient for the progression of invasive cervical cancer in all women[14].

This implies that other cofactors, including environmental influences (including smoking, chlamydia trachomatis infection and the use of oral contraceptives), are involved in it[15] and hereditary variables that potentially raise the chance of invasive cervical cancer development[16]. HPV exposure is a central element associated with multiple risk factors for cervical cancer[17,18]. HPV induced precursor lesions, acquired through sexual transmission, can take as long as 20years to develop into invasive cervical cancer[19]. Cervical cancer is associated with various risk factors, such as engaging in sexual activities before reaching sixteen years of age, having multiple sexual partners, smoking, high parity and low socioeconomic status-many of which are linked

to reproductive, sexual and behavioral factors[20,21]. The Global Cancer Observatory reports show that in 2020, In a recent global estimate, cervical cancer caused 341,831 deaths and 604,127 new cases. The global annual death rate due to cervical cancer stands at 7.2 per 100,000 women. Whereas the age-standardized incidence rate (ASR) was 13.3 cases per 100,000 women. Asia had the highest overall incidence and death rates (over 58%), with an annual rate of 40 cases and 28.6 deaths per 100,000 women. Africa has the highest age-standardized incidence and mortality rate for cervical cancer[22]. Genetic variation is a recognized contributor to cervical cancer development, among other risk factors. Paired box 8 is a gene essential for growth and development. As a transcription factor, it binds to a specific sequence of DNA to control the expression of other genes. This transcription factor also plays a role in cell division and proliferation. It contributes to essential physiological processes and is significantly associated with tumorigenesis[23]. This study examined the association between three transcription factor gene polymorphisms-PAX8 (rs10175462), PBX2 (rs2856437) and TERT (rs2853669)-with cervical cancer susceptibility in our population by genotypic analysis. The results demonstrated a notable genotype-specific relationship across all three genes, underscoring their potential as genetic biomarkers in cervical carcinogenesis.

The PAX8 rs10175462 polymorphism exhibited a protective correlation with the heterozygous GT genotype (OR = 0.46), whereas the homozygous mutant TT genotype was associated with an almost threefold increased risk of cervical cancer (OR = 2.91). The aggregated GT+TT genotypes exhibited no significant correlation, indicating separate genotype-specific effects. PAX8 is crucial for epithelial development in the female reproductive system and its abnormal expression has been associated with gynecologic tumors, including ovarian and endometrial malignancies[24]. Our results substantiate the functional significance of PAX8 variants in cervical cancer, consistent with genome-wide data indicating that the rs10175462 locus within PAX8 is a notable susceptibility variant in both UK Biobank and Finnish populations (OR ≈ 0.87 per T allele, $p = 1.07 \times 10^{-9}$; replicated with p = 0.015)[25]. The heterozygous GT genotype seems to confer protection, whereas the TT genotype markedly elevates risk, underscoring the complexity of PAX8 regulation. This dual role exemplifies the principle of heterozygote advantage; wherein heterozygous genotypes may provide superior fitness compared to either homozygote. This behavior, however uncommon, is substantiated by evolutionary theory[26].

The analysis of the PBX2 rs2856437 polymorphism indicated that the heterozygous GA genotype considerably increased illness susceptibility (OR=1.88), whereas the AA mutant genotype had a minimal and statistically insignificant effect. The GA+AA genotypes, however, exhibited a robust association with the risk of cervical cancer. PBX2 is a member of the TALE family of transcription factors that interacts with HOX proteins to modulate oncogenic transcriptional processes[27]. Our findings corroborate large-scale genomic research indicating that PBX2 mutations, especially rs2856437, are strongly linked to heightened cervical cancer risk (OR = 1.52) in separate case-control analyses[6]. The insignificance of the AA genotype in our dataset may arise from the restricted number of people possessing this variant, reflecting difficulties previously highlighted in candidate gene research regarding statistical power and allele frequency[6].

The TERT rs2853669 polymorphism's heterozygous AG genotype was linked to an almost two-fold elevated risk of cervical cancer (OR= 2.57). The homozygous GG genotype seemed to provide a protective effect. The AG + GG genotypes exhibited a notable increase in susceptibility. These findings are supported by a previous study including South Indian women, which particularly investigated the relationship between rs2853669, TERT promoter mutations and HPV status in cervical cancer[28]. Moreover, extensive meta-analyses indicate that rs2853669 is significantly associated with heightened cancer risk, especially in Asian populations, while exhibiting a negligible or lesser correlation in Caucasians. The variation in risk linkages among ethnic groups highlights the necessity of doing ethnicity-focused research to comprehend population-specific genetic factors[29,30].

5. Conclusion

This study demonstrates that specific single-nucleotide polymorphisms (SNPs) in the *PAX8* (rs10175462), *PBX2* (rs2856437) and *TERT* (rs2853669) genes are significantly associated with cervical cancer susceptibility in the studied population. The mutant (TT) genotype of *PAX8* rs10175462, the heterozygous (GA) genotype of *PBX2* rs2856437 and the heterozygous (AG) genotype of *TERT* rs2853669 consistently showed strong correlations with elevated disease risk, with some variants conferring more than a two-fold increase in susceptibility. Furthermore, family history emerged as a major risk factor, showing a 5-fold higher likelihood of disease, while smoking status, age distribution and gene–environment interactions also demonstrated significant associations. These findings suggest that *PAX8*, *PBX2* and *TERT* polymorphisms could serve as potential genetic biomarkers for early identification of individuals at high risk of cervical cancer, facilitating targeted prevention and personalized management strategies.

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7. Conflict of Interest

The authors declare that they have no competing interests.

8. Authors Contribution

Authors 1 and 2: contribute equally in writing, proof reading, analysis and lab work

Authors 2 and 3 conceptualize and design whole project.

9. Availability of data and materials

All the data and material are available. Due to secrecy and privacy of patient's data will be provided on journal's demand. In case of any demand please contact with Corresponding author.

10. Consent for publication

Not applicable.

11. Ethical Approval and Consent to participate

Before the start of project ethical approval was taken from the ethical committee Institute of Molecular Biology and Biotechnology and collaborating hospitals letter no. No. SUS/BIO-SGD/141 dated 19 June 2024. Before the collection of blood samples, consent Performa was sign by each patient.

12. Funding

NA.

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Supplementary Table1: Primers for PAX8, PBX2 and TERT gene SNPs with product size and annealing temperatures.

Name of SNPs	Primer Sequence	Product Size (bp)	Annealing Temp. (°C)
rs10175462		144	58
*F	TCCTCGCTATGAATTCCATCTCC		
**R	TTCAAGGTGTAGGGGGAGCTA		
rs2856437		138	52
*F	AGGGAGGTTTGAGAGGGATCA		
**R	CCAGCAAACCCCGTTCACAT		
2853669		103	60
*F	GAAGGTGAAGGGGCAGGAC		
**R	CGGGCTCCCAGTGGATTC		

*Forward, **Reverse