Polymorphic variants \textit{INSIG2} rs6726538, \textit{HLA-DRB1} rs9272143, and \textit{GCNT1P5} rs7780883 contribute to the susceptibility of cervical cancer in the Bangladeshi women

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**ABSTRACT**

**Objective:** Cervical cancer is a gynecological health problem, affecting nearly 500,000 women each year worldwide. Genome-wide association studies have revealed multiple susceptible genes and their polymorphisms for cervical carcinoma risk. We have carried out this case-control study to investigate the association of \textit{INSIG2} rs6726538 (A; T), \textit{HLA-DRB1} rs9272143 (T; C), and \textit{GCNT1P5} rs7780883 (G; A) with cervical cancer.

**Methods:** The present study recruited 234 cervical cancer patients as cases and 212 healthy females as controls. We have applied the tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) method for genotyping.

**Results:** The SNP rs6726538 was significantly associated with increased risk of cervical cancer in all genetic models (AT vs. AA: OR = 3.30, 95% CI = 2.19–4.97, \(p < 0.0001\); TT vs. AA: OR = 8.72, 95% CI = 3.87–19.7, \(p < 0.0001\); AT+TT vs. AA: OR = 3.87, 95% CI = 2.61–5.73, \(p < 0.0001\); T vs. A: OR = 2.97, 95% CI = 2.20–4.01, \(p < 0.0001\)) except the recessive model which showed a significantly reduced risk (TT vs. AA+AT: OR = 0.20, 95% CI = 0.09–0.44, \(p = 0.0001\)). rs9272143 showed significantly reduced risk for the additive model 1, dominant model, and allelic model (TC vs. TT: OR = 0.46, 95% CI = 0.31–0.70, \(p = 0.0004\); TT+TC vs. TT: OR = 0.47 95% CI = 0.32–0.70, \(p = 0.0002\); C vs. T: OR = 0.56, 95% CI = 0.40–0.78, \(p = 0.0006\), respectively). The third variant, rs7780883, was significantly associated with increased risk in additive model 2, dominant, and allelic models (AA vs. GG: OR = 5.08, 95% CI = 2.45–10.5, \(p < 0.0001\); GA+AA vs. GG: OR = 1.54, 95% CI = 1.06–2.24, \(p = 0.0237\); A vs. G: OR = 1.88, 95% CI = 1.34–2.52, \(p < 0.0001\), consecutively), whereas recessive model reduced the risk of cervical cancer (AA vs. GG+GA: OR = 0.20, 95% CI = 0.09–0.41, \(p < 0.0001\)). Other models of these SNPs
1 INTRODUCTION

Cervical cancer (CC) is the world’s fourth most common type of female malignancy that represents a burden on global health considering incidence and mortality. CC constitutes almost 6.6% of all cancers in women, and around 570,000 new cases were recorded in 2018. CC is the second leading cause of death from cancer in women between the ages of 20 and 39, causing nine deaths per week in this age group. In addition, it is the second most frequent cause of death among women aged 15 to 44 years old. In Bangladesh, cervical cancer is the second most common cancer in women, with a total of 11,956 women diagnosed each year in Bangladesh, and over 6582 of them die each year.

Human papillomavirus (HPV) is thought of as one of the main precursors of cervical cancer induced by persistent infection. Despite the availability of some vaccines those work against some different types of HPV, reducing the risk of developing cervical cancer, individuals’ genetic susceptibility to the disease may control the immune response. During the early stages, HPV-associated cervical cancer progresses asymptptomatically. In most cases, the virus may remain undetected if not tested in time and starts tumor formation, leading to cervical cancer development. Screening within the ages of 30 to 49 years may prevent the development, and follow-up diagnosis and treatment increase the possibility to remove cancer in the early stage. However, research has demonstrated that HPV alone does not entirely clarify cervical malignancy; instead, various cofactors are related to the progression of this carcinogenicity. Additional host factors, such as women’s age, number of abortions, first delivery age, multiple childbirths, multiple sexual partners, oral contraceptive pills, immune suppression, smoking of cigarettes, and poor socioeconomic condition, are also responsible for the development of CC. Moreover, multiple genetic factors play a significant role in the progression of developing cervical carcinoma.

INSIG2 (insulin-induced gene 2) is an endoplasmic reticulum (ER) protein that regulates or blocks the proteolytic activation of sterol regulatory element-binding proteins (SREBPs), transcription factors that may trigger fatty acid and cholesterol synthesis. Studies explicated that INSIG2 was associated with obesity. It was also reported to be a biomarker for the development of colon cancer and pancreatic cancer. This evidence suggests that INSIG2 might show an emergent contribution to the progression of cancers. However, no previous studies are suggesting the involvement of INSIG2 in cervical cancer except a GWAS study. Intergenic rs6726538 (A; T) is located on chromosome 2 (2q14.2), and the A allele of rs6726538 is documented to be associated with the progression of cervical cancer (OR = 1.20, 95% CI = 1.07–1.35 and p = 7.14 × 10^−3 for the risk allele A).

SNP rs9272143 (T; C) is located in the class II region (major histocompatibility complex, MHC region) of chromosome 6 (p-arm, 6p21.32) between HLA-DRB1 and HLA-DQBI and associated with cervical cancer. More specifically, rs9272143 is positioned at 43.19 kb upstream of HLA-DRB1 and 4.38 kb upstream of HLA-DQA1. HLA-DRB1 is a member of the HLA class II β-chain paralogs that encodes the β-chain of the HLA-DR, which is a peptide-antigen receptor. It is commonly found in the antigen-presenting cells (APCs) of squamous epithelia in the cervix and Langerhans cells (LC). It was observed that HLA-DRB1 plays a major role in the cell-mediated immune response by presenting antigens to CD4+ helper T cell, which after activation, secretes different small proteins or cytokines.

We have tried to keep the focus on the rs9272143 variant concerning the progression of cervical cancer as HLA-DRB1 expression is found in the cervix.

rs7780883 (G; A) SNP of glucosaminyl (N-acetyl) transferase 1 pseudogene 5 (GCNT1P5) is located on an intergenic region of chromosome 7, and a genome-wide association study (GWAS) revealed that the A allele of rs7780883 is associated with the development of cervical cancer (OR = 3.28, 95% CI = 1.97–5.5 and p = 2.49 × 10^-6 for the risk allele A). Therefore, we have tried to find out the risk susceptibility of this variant with cervical carcinogenesis.

GWAS with these three different novel variants (rs6726538 of INSIG2, rs9272143 of HLA-DRB1, and rs7780883 of GCNT1P5) imposed the probability of cervical cancer progression. Again, due to geographical or ethnic differences, the rate and extent of disease occurrences in different world...
regions are observed. Even the variation can be found in the same ethnicity. So, we tried to find out the association of cervical cancer with these SNPs in patients from various regions of Bangladesh. To add, we choose the tetra-primer amplification refractory mutation system–polymerase chain reaction (T-ARMS–PCR) method to perform the study that works with simple gel electrophoresis following the PCR run.

2 | MATERIALS AND METHODS

2.1 | Study design and sample recruitment

We carried out the present case-control study in 234 cervical cancer patients recruited as cases from the National Institute of Cancer Research and Hospital, Dhaka, Bangladesh. Two hundred and twelve healthy volunteers were recruited as controls from the different parts of the country. Histologically diagnosed cervical cancer was confirmed in the patients, and controls were chosen by matching age with the cases. The exclusion criteria for both the case and the controls are: (a) those under the age of 21; (b) those unable to provide relevant data; (c) those suffering from comorbidities or chronic diseases; and (d) those who declined to participate in the study. All participants signed and agreed to participate in this study, also consenting to the consequent publication of the results, according to the written consent form. In terms of lifestyle and sociodemographic factors associated with an increased risk of cervical cancer, each patient and control subjects were interviewed. The tumor location, tumor stage, histological type, and condition of lymph nodes were collected from the patients’ medical records. The study protocol and consent form were reviewed and approved by the ethics committee of the National Institute of Cancer Research and Hospital, Bangladesh (NICRH/Ethics/2019/447). The research was carried out following the principles of conducting research on human subjects according to the Helsinki Declaration and its further correction.

This study was conducted at the Pharmacogenomics and Molecular Biology Lab, Department of Pharmacy, Noakhali Science and Technology University, Bangladesh.

2.2 | Collection and storage of blood, isolation, and quantification of genomic DNA

Peripheral blood (around 3 ml) was collected from individuals from both the groups and transferred to a plastic tube containing EDTA (ethylenediaminetetraacetic acid) and stored at −80°C until the extraction of DNA. Genomic DNA was isolated using “FavorPrep” DNA extraction mini kit following the protocol book supplied with the kit. The concentration of extracted DNA was measured using a microvolume spectrophotometer (Genova Nano, Jenway). The absorption ratio of 260 nm and 280 nm was used to assess the genomic DNA purity.

2.3 | Primer design and genotyping

Genotyping of SNPs was completed employing the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method. A few online programming were utilized for primer design. Four primers, such as forward outer, reverse outer, forward inner, and reverse inner, were designed to amplify the desired allele (Table 1). PCR premix was formulated by adding EmeraldAmp GT

| SNPs    | Primers | Sequence (5’−3’) | Allele | Amplicon size (bp) |
|---------|---------|-----------------|--------|--------------------|
| rs6726538 | FI      | CAACCCCATCCCCCTTGCTATTTATT | A      | 261                |
|         | RI      | GAACACTGATTATGTGATAGTCTTTCTGTAT |        | 231                |
|         | FO      | GAGTAGCTGGGACTACAAGCACAACACTA | T      | 432                |
|         | RO      | CTCACTTTCCACAAACTTGAAGGAAGA |        | 432                |
| rs9272143 | FI      | CATAAAAAAATCTGACAGATAAACGCG | T      | 222                |
|         | RI      | ATGCTGAAAAACAAAAATTTTTTGGAGA |        | 177                |
|         | FO      | ACCTATTGATGCTACAGAGATGTGAGGG | C      | 342                |
|         | RO      | AATGATAATAACATCATGCTTTGGGCTG |        | 342                |
| rs7780883 | FI      | TACACTCTGAAGCTGACTGGCA | G      | 143                |
|         | RI      | TTGTATTTCAAGGGTATACCTGGACTC |        | 201                |
|         | FO      | ATAAAAACATGTCAAAAATTAAGGAAAGGG | A      | 288                |
|         | RO      | TGATATTCTTGGGCGATAGTCCATCT |        | 288                |

Abbreviations: FI, Forward inner; FO, Forward outer; RI, Reverse inner; RO, Reverse outer.
PCR Master Mix, nuclease-free water, MgCl₂, primers at a specified concentration. For instance, the volume of outer primers (forward outer and reverse outer) was 1.5 μl, and inner primers (forward inner and reverse inner) was 2.5 μl for a 120 μl PCR master mix solution (12 samples; 10 μl/reaction). The PCR was then performed by adding DNA sample with the premix (10 μl) at the required concentration. Next, the PCR products were analyzed by gel electrophoresis (1% agarose gel) to get the confirmation of the DNA bands of specific alleles staining with ethidium bromide.

2.4 Validation process of tetra-primer ARMS–PCR method

Method validation was performed for selecting a suitable annealing temperature for the selected SNPs to get the desired DNA fragments. The melting temperatures of primers were calculated manually. We selected 64°C, 51°C, and 64°C temperatures consecutively as our desired annealing temperature for SNPs INSIG2 rs6726538, HLA-DRB1 rs9272143, and GCNT1P5 rs7780883. For rs6726538 SNP, at 64°C temperature, we detected 432 bp, 261 bp, and 231 bp size fragments (Figure 1), whereas for rs9272143 SNP, at 51°C temperature, we detected 342 bp, 222 bp, and 177 bp size fragments (Figure 2) and for rs7780883 SNP, at 64°C temperature, we detected 288 bp, 201 bp, and 143 bp size fragments (Figure 3). The details of PCR conditions for rs6726538, rs9272143, and rs7780883 SNPs with fragment size are illustrated in Table 2.

2.5 Statistical calculation

SPSS software package, version 23.0 (IBM, Armonk, NY, USA) was applied for calculating all statistical data. Chi-square test (χ²), odds ratio (OR), and their 95% confidence intervals (CI) and Hardy–Weinberg Equilibrium (HWE) were calculated. The genotype and allelic frequencies were reported as the percentage. For all analyses, a statistically significant value was considered at \( p < 0.05 \). Bonferroni correction was performed to correct the \( p \)-values, and a \( p \)-value of <0.0033 (for three SNPs and five genetic models for each SNP, \( p = 0.05/5 \times 3 = \)) after the Bonferroni correction was considered statistically significant.29

3 RESULTS

3.1 Distribution of demographic data between subjects

The demographic characteristics of patients and control subjects are reported in Table 3. The minimum and maximum ages of patients were 35 and 80 years (mean age 57.5 years), and healthy controls were 30 and 75 years (mean age 52.5 years), respectively. The frequencies of patients under 45 years and between 45 and 60 years were 33.33% and 58.1%, respectively, whereas for controls, these were 32.55% and 62.73%, consecutively. The percentage of both patients and controls aged over 60 years was 8.5% and 4.72%, respectively. Besides, most of the patients suffered from stage IIB (38.57%) of cervical cancer. The percentage of stage IIIB cervical carcinoma patients was also high, comprising of 24.29%. The frequency of grade II tumor was higher (42.73%) compared to grades I and III. The negative lymph
nodes found in the patients were higher than the positive (90.6% vs. 9.4%) lymph nodes. However, no mentionable data were found for smoking status and alcohol consumption in the patients.

### 3.2 Genotype distribution and contribution of variants to cervical cancer risk

The genotypic and allelic frequencies and the association of different genetic models of *INSIG2* rs6726538, *HLA-DRB1* rs9272143, and *GCNT1P5* rs7780883 with Bonferroni correction are illustrated in Table 4.

With regard to the rs6726538 (A; T) polymorphism in *INSIG2* for the case group, the frequencies of the alleles A and T were 57.7% and 42.31%, respectively, whereas for the control group, the frequencies of the alleles A and T were 80.19% and 19.81%, respectively. The frequency of genotypes AA, AT, and TT in cases were 31.62%, 52.14%, and 16.24%, individually and control frequencies were 64.15%, 32.08%, and 3.78%, consecutively. Women carrying AT genotype showed 3.30 times higher risk for the development of cervical cancer compared to the wild-type AA, and this result is statistically significant (OR = 3.30, 95% CI = 2.19–4.97, p < 0.0001). Like the same way, women carrying TT genotype showed 8.72 times significantly higher risk of developing cervical cancer compared to AA genotype carrying women (OR = 8.72, 95% CI = 3.87–19.7, p < 0.0001). Dominant model carriers (women carrying both AT and TT genotypes) had 3.87 times higher risk of developing cervical cancer (OR = 3.87, 95% CI = 2.61–5.73, p < 0.0001), and it is statistically significant. Women carrying T allele (minor) also showed 2.97 times higher risk of developing cervical cancer, and the data are statistically significant (T vs. A: OR = 2.97, 95% CI = 2.20–4.01, p < 0.0001). Only the recessive model carriers (TT vs. AA+AT) had a protective effect in comparison to AA+AT genotype, and these data are statistically significant (OR = 0.20, 95% CI = 0.09–0.44, p = 0.0001). It was found that all associations were statistically significant after performing Bonferroni correction (p < 0.003).

In case of the *HLA-DRB1* rs9272143 (T; C) variant, the frequencies of TT, TC, and CC genotypes were 72.65%, 24.79%, and 2.56%, consecutively in cases, whereas controls had 55.66%, 40.56%, and 3.77%, respectively. The frequency of minor allele C allele was 15.09% in cases and 24.06% in controls. rs9272143 SNP showed reduced risk association for cervical cancer in additive model 1, dominant model, and allele model, and the associations withstand even after Bonferroni correction (TC vs. TT: OR = 0.46, 95% CI = 0.31–0.70, p = 0.0004; TC+CC vs. TT: OR = 0.47 95% CI = 0.32–0.70, p = 0.0002; C vs. T: OR = 0.56, 95% CI = 0.40–0.78, p = 0.0006). No association of cervical cancer was found with additive model 2 and recessive model (CC vs. TT: OR = 0.52, 95% CI = 0.18–1.54, p = 0.0899; recessive model: OR = 1.49, 95% CI = 0.51–4.37, p = 0.47).

The third variant rs7780883 (G; A) of *GCNT1P5* exhibited a significant association in three genetic models for the increased risk of cervical cancer in the studied sample (additive model 2, AA vs. GG: OR = 5.08, 95% CI = 2.45–10.5, p < 0.0001; dominant model, GA+AA vs. GG: OR = 1.54, 95% CI = 1.06–2.24, p = 0.0237; allele model, A vs. G: OR = 1.88, 95% CI = 1.34–2.52, p = <0.0001) that was also significant after Bonferroni correction except for the dominant model. Women carrying GA and AA genotypes

| SNP      | PCR conditions | No. of cycles | Size of PCR products (bp) | Genotype |
|----------|----------------|---------------|----------------------------|----------|
| rs6726538 | 95°C for 5 min  | 35 cycles     | NH: 261,432               | AA       |
|          | 95°C for 1 min  |               | HE: 231,261               | AT       |
|          | 64°C for 30 s   |               | MH: 231,432               | TT       |
|          | 72°C for 30 s   |               |                           |          |
|          | 72°C for 10 min |               |                           |          |
| rs9272143 | 95°C for 5 min  | 35 cycles     | NH: 222,342               | TT       |
|          | 95°C for 1 min  |               | HE: 177,222               | TC       |
|          | 51°C for 30 s   |               | MH: 177,342               | CC       |
|          | 72°C for 1 min  |               |                           |          |
|          | 72°C for 10 min |               |                           |          |
| rs7780883 | 95°C for 5 min  | 35 cycles     | NH: 143,288               | GG       |
|          | 95°C for 1 min  |               | HE: 143,201               | GA       |
|          | 64°C for 30 s   |               | MH: 201,288               | AA       |
|          | 72°C for 1 min  |               |                           |          |
|          | 72°C for 10 min |               |                           |          |

Abbreviations: HE, Heterozygote; MH, Mutant Homozygote; NH, Normal Homozygote.

**TABLE 2** PCR conditions for rs6726538, rs9272143, and rs7780883 SNPs with fragment size
is the major cause of developing cervical cancer, almost 70% to 90% of patients can recover from this infection. However, several host factors are responsible for the high prevalence of this disease, as well as the mortality caused by it. Poor development of the immune system, environmental factors, as well as genetic variations or alterations lead to high susceptibility. GWAS have been carried out to correlate the genetic polymorphisms or variability responsible for the predisposition of cervical cancer in females. Moreover, interpreting the function of genetic polymorphisms in cervical carcinoma leads to the discovery of personalized medicine. Due to the variable genetic polymorphisms between different ethnic groups, only the successful identification of these polymorphisms and quantification of gene expression can help to manage cancer. Our study found the association of INSIG2 rs6726538 (A; T), HLA-DRB1 rs9272143 (T; C), and GCNT1P5 rs7780883 (G; A) polymorphisms with cervical cancer risk in the Bangladeshi population.

As far as we know, more than 30 susceptible genes have been reported for their crucial role in the progression of cervical malignancy, namely HLA B/C, tumor necrosis factor-α (TNF-α), interleukin 10 (IL10), interleukin 12 (IL12), cytochrome P450 1A1 (CYP1A1), p53, p16, p21, poly [ADP-ribose] polymerase 1 (PARP1), BRCA1-interacting protein 1 (BRIP1), cytotoxic T lymphocyte antigen 4 (CTLA4), X-ray repair cross-complementing protein 1 (XRCC1), Caspase 8, C-C chemokine receptor type 2 (CCR2), exonuclease 1 (EXO1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), fas ligand (FASLG), fas receptor (FASR), HOX transcript antisense RNA (HOTAIR), interferon-γ (IFN-γ), murine double minute 2 (MDM2), BH3-interacting death agonist (Bid), Tap2TLR9, and methylenetetrahydrofolate reductase (MTHFR). INSIG2 is an important ER protein that possibly inhibits the processing of SREBPs through binding to the cleavage activating protein (SCAP) in a well-controlled fashion and preventing the proteolytic mechanism of SREBPs.

The expression of INSIG2 has been associated with the progression of colorectal cancer metastasis and its outcome. An investigation has explicated the association of INSIG2 with pancreatic cancer. This evidence suggests the probability of this gene with cervical cancer. We have investigated and found the association of rs6726538 of INSIG2 with the risk of cervical cancer in the Bangladeshi population. Our results demonstrated that all the genetic models were associated with cervical malignancy, and the associations were significant even after the Bonferroni correction. The frequency of minor allele T was higher in the patient group (42.31%) than in the controls (19.81%). We have observed that the frequency of AT (52.14%) and TT (16.24%) genotypes in the patient group was also higher than the control group.

### 4 | DISCUSSION

Cervical cancer (CC) is responsible for extreme mortality among women throughout the world. Though HPV infection showed 1.08 times higher risk (GA vs. GG: OR = 1.08, 95% CI = 0.72–1.62, \( p = 0.7245 \)) compared to wild allele GG, and the association was found not to be statistically significant. The recessive model showed a reduced risk for cervical cancer development, and it was statistically significant even after Bonferroni correction (AA vs. GG+GA: OR = 0.20, 95% CI = 0.09–0.41, \( p < 0.0001 \)). The frequencies of A allele (minor) were 35.47% and 22.64% in the cases and controls, respectively. The percentages of GG, GA, and AA genotypes in cases were 48.72%, 31.62%, 19.66%, respectively, and in controls, these values were 59.43%, 35.85%, and 4.72%, consecutively.

### TABLE 3 Demographic characteristics of the patients and controls

| Variables              | Cases, \( n = 234 \) (%) | Controls, \( n = 212 \) (%) |
|------------------------|---------------------------|-------------------------------|
| Age (Years)            |                           |                               |
| <45                    | 78 (33.33%)               | 69 (32.55%)                   |
| 45–60                  | 136 (58.1%)               | 133 (62.73%)                  |
| >60                    | 20 (8.57%)                | 10 (4.72%)                    |
| Tumor Grade            |                           |                               |
| I                      | 80 (34.19)                | N/A                           |
| II                     | 100 (42.73)               | N/A                           |
| III                    | 54 (23.08)                | N/A                           |
| Histological Type      |                           |                               |
| SQC                    | 108 (46.15)               | N/A                           |
| Adenocarcinoma         | 53 (22.65)                | N/A                           |
| SCC                    | 10 (4.27)                 | N/A                           |
| Endometrioid           | 21 (9.87)                 | N/A                           |
| Other                  | 42 (17.95)                | N/A                           |
| Tumor Stage            |                           |                               |
| IIB1-IIB2              | 26 (10.95%)               | N/A                           |
| IIA                    | 9 (3.81%)                 | N/A                           |
| IIB                    | 90 (38.57%)               | N/A                           |
| IIIA                   | 33 (14.29%)               | N/A                           |
| IIIB                   | 57 (24.29%)               | N/A                           |
| IVA-IVB                | 19 (8.095%)               | N/A                           |
| Lymph Nodes            |                           |                               |
| Negative               | 212 (90.6)                | N/A                           |
| Positive               | 22 (9.4)                  | N/A                           |
| Smoking status         | Not found                 | N/A                           |
| Alcohol consumption    | Not found                 | N/A                           |

Abbreviations: SCC, Serous cystadenocarcinoma; SQC, Squamous cell carcinoma.
GWAS and other genetic association studies have been investigated with the human leukocyte antigen (HLA) loci in cervical neoplasia. Studies have reported that the haplotype HLA-B*0702-DRB1*1501/HLA-DQB1*0602 increases the risk of cervical carcinoma, whereas the haplotype HLA-B*1501/HLA-DRB1*1301/HLA-DQA1*0103/HLA-DQB1*0603 protects from the risk of disease progression. A study of 84 Finnish individuals showed that the rs9272143 variant was a cis-expression quantitative trait locus (eQTL), which alters the expression of HLA-DRB1 in fatty tissue, and the C allele significantly increases the expression of HLA-DRB1. Leo et al. also explicated a strong correlation of cervical cancer with both risk and protective HLA haplotypes. These are ascertained by the presence of amino acids of HLA-DRB1 at positions 13 and 71 in pocket 4 and for HLA-B at position 156. However, on account of rs9272143 polymorphism, we have found that the TC, TC+CC genotype, and C allele act like a protective factor in Bangladeshi cervical cancer patients. These associations were also significant after the Bonferroni correction. We have also reported that the AA mutant homozygote frequency is higher in patients than in controls. The frequency distribution showed a lower frequency of minor allele C in cases compared to healthy controls.

The third variant of our study is rs7780883 SNP in GCNT1P5 gene, which is an intergenic variant and located on chromosome 7. GWAS have reported this variant as a risk factor for cervical cancer development. However, to the best of our knowledge, no previous association study or case-control study was conducted on this polymorphism with the risk of cervical malignancy. Consequently, this is the first case-control analysis of rs7780883 in the world and in Bangladesh to provide proof of association with cervical cancer, except for a GWAS study. Our study demonstrates that the additive model 2, the dominant model, and the minor allele A increased the risk of cervical cancer significantly.

| SNPs            | Genotype | Cases | %  | Controls | %  | Genetic models | OR    | 95% CI     | p-value  |
|-----------------|----------|-------|----|----------|----|----------------|-------|-----------|----------|
| rs6726538       | Genotype | AA    | 74 | 31.62    | 136| 64.15          | 3.30  | 2.19–4.97 | <0.0001  |
|                 |          | AT    | 122| 52.14    | 68 | 32.08          | 8.72  | 3.87–19.7 | <0.0001  |
|                 |          | TT    | 38 | 16.24    | 8  | 3.78           | 0.20  | 0.09–0.44 | 0.0001  |
|                 | Allele   | A     | 270| 57.70    | 340| 80.19          | 1     |           |          |
|                 |          | T     | 198| 42.31    | 84 | 19.81          | 2.97  | 2.20–4.01 | <0.0001  |
| rs9272143       | Genotype | TT    | 170| 72.65    | 118| 55.66          | 0.46  | 0.31–0.70 | 0.0004  |
|                 |          | TC    | 58 | 24.79    | 86 | 40.56          | 0.47  | 0.32–0.70 | 0.0002  |
|                 |          | CC    | 6  | 2.56     | 8  | 3.77           | 1.49  | 0.51–4.37 | 0.47    |
|                 | Allele   | T     | 398| 85.78    | 322| 75.94          | 1     |           |          |
|                 |          | C     | 70 | 15.09    | 102| 24.06          | 0.56  | 0.40–0.78 | 0.0006  |
| rs7780883       | Genotype | GG    | 114| 48.72    | 126| 59.43          | 1.08  | 0.72–1.62 | 0.7245  |
|                 |          | GA    | 74 | 31.62    | 76 | 35.85          | 5.08  | 2.45–10.5 | <0.0001  |
|                 |          | AA    | 46 | 19.66    | 10 | 4.72           | 0.20  | 0.09–0.41 | <0.0001  |
|                 | Allele   | G     | 302| 64.53    | 328| 77.36          | 1     |           |          |
|                 |          | A     | 166| 35.47    | 96 | 22.64          | 1.88  | 1.34–2.52 | <0.0001  |

*p < 0.05 was considered as statistically significant (bold), whereas * indicates significant after Bonferroni correction (p < 0.0033).
whereas the recessive model significantly reduced cervical cancer risk. All the associations withstand after Bonferroni correction except the dominant model. The frequency of minor allele A was higher (35.47%) in cancer patients than in the healthy controls.

Our present study also found that the patients aged between 45 and 60 years are at higher (58.1%, 95%Cl = 51.50–64.50, $p < 0.0001$) risk than other age groups in Bangladesh. Moreover, the frequency of patients with tumor stages IIB and IIIB were higher than other stages of tumor comprising 38.57% (95%Cl = 32.30–45.13, $p < 0.0001$) and 24.29% (95%Cl = 18.94 to 30.30, $p < 0.0001$), respectively, for the patients. We have also validated tetra-primer ARMS-PCR for the genotyping of these variants for the first time.

To mention, besides the strengths of our study findings, it had some lacking as our sample size was not appreciable. In this case, although the sample was somewhat small, we found the association of cervical cancer with all the three SNPs after performing the Bonferroni correction.

5 | CONCLUSION

Our study summarizes that INSIG2 rs6726538 (A; T), HLA-DRB1 rs9272143 (T; C), and GCNT1P5 rs7780883 (G; A) polymorphisms are associated with cervical cancer development in the Bangladeshi population. We have identified the association of these variants with cervical carcinoma for the first time in Bangladesh. However, we suggest further study on a large scale on different cohorts to find in detail genotype–phenotype associations of these variants.

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ETHICAL DECLARATIONS

The research protocol and consent form were reviewed and approved by the ethics committee of the National Institute of Cancer Research and Hospital (NICRH). The ID of ethical approval was NICRH/Ethics/2019/447.
DATA AVAILABILITY STATEMENT
The datasets used in this study are available from the corresponding author on a reasonable request.

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