RESEARCH ARTICLE

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Analysis of *E-cadherin* (*CDH1*) Gene Polymorphism and Its Association with Cervical Cancer Risk in Bangladeshi Women

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Abstract

Background: *E-cadherin (CDH1)*, a tumor suppressor gene, encodes a transmembrane glycoprotein that helps in maintaining squamous epithelium integrity of the cervix. We aimed to investigate the association between -160C/A genetic polymorphism in *CDH1* and the risk of cervical cancer in Bangladeshi females. **Method:** The present case-control study included 117 cervical cancer cases and 147 age-matched controls. The genomic DNA was extracted from peripheral blood and genotyped by using PCR–RFLP analysis. **Results***:* Genotyping results demonstrated that the occurrences of normal homozygous (-160C/C), heterozygous (-160C/A) and variant homozygous (-160A/A) genotypes were 64.10, 27.35 and 8.55% in cases, and 77.55, 19.73 and 2.72% in controls, respectively. Compared to normal C/C genotype, variant A/A and combined (C/A+A/A) or 'any A' genotypes exhibited 3.80-fold (95% CI=1.150-12.561, P=0.029) and 1.93-fold (95% CI=1.126-3.323, P=0.017) increased risk of cervical cancer development. The -160C allele was found to be positively linked to cervical cancer incidence and raised the risk by 1.81-fold (OR= 1.814, 95% CI=1.152-2.857, p=0.01). Moreover, women carrying -160A/A variant homozygosity along with an early marital history (<18 years) were more susceptible to cervical cancer development (χ^2 =6.605, p=0.037). **Conclusion:** The study suggests that the (A/A) and combined (C/A +A/A) genotypes are associated with greater risk of cervical cancer in Bangladeshi women.

Keywords: E-cadherin1- cervical cancer- SNP- genetic polymorphism- Bangladesh

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Introduction

Cervix is located at the lowermost part of the uterus that connects vagina and uterus. Cancer occurring in the cells of the cervix is termed as cervical cancer. In 2018, there were an estimated five hundred seventy thousand new cervical cancer cases and three hundred eleven thousands deaths, worldwide (Bray et al., 2018; Sung et al., 2021). However, approximately 90% of the deaths due to cervical cancer in women occur in developing countries (Cohen et al., 2019). This malignancy is thought to be a multifactor disease. A number of molecular studies have suggested high-risk HPV (human papillomavirus) subtypes to be linked to the onset of cervical cancers in women (Cohen et al., 2019; Crosbie et al., 2013; Walboomers et al., 1999; zur Hausen, 1996). Recent genome-wide association studies have shown that genetic polymorphisms in immune responsive genes affect the susceptibility to the HPV infection and thus play a crucial role in cervical carcinogenesis (Hildesheim and Wang, 2002; Wang and Hildesheim, 2003). However, apart from the HPV infection, other factors are also supposed to be associated with cervical cancer as every HPV-infected female does not develop cervical cancer (Hariri et al., 2011; Ramond et al., 1992) and also most of the patients with HPV-positive cervical carcinomas do not progress to the invasive cancer stages (Holowaty et al., 1999). Genetic polymorphisms in various crucial genes have been described in scientific literatures demonstrating their association with cervical cancer risk (Alsbeih et al., 2017; Apu et al., 2020; Liu et al., 2019).

E-cadherin (*CDH1*), one of the most important tumor invasion suppressor genes, is positioned on chromosome 16q22.1 (Kangelaris and Gruber, 2007) which encompasses an extracellular domain with five cadherin repeats, a tinier transmembrane segment and a short cytoplasmic domain (Ringwald et al., 1987). The *CDH1* gene encodes a 120 kDa cadherin transmembrane glycoprotein, *CDH1*, which is expressed in nearly all mammalian epithelial cells (Kucharczak et al., 2008; Martinez-Rico et al., 2010) and the level of *CDH1* expression have been shown to be increased in cancer tissues including anthracosis patients (Izadian et al., 2017).

Cadherins are a class of calcium-dependent cell

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adhesion molecules that exert a key role in the formation and preservation of epithelial integrity, cellular polarity, and tissue architecture (Suzuki and Takeichi, 2008; Takeichi et al., 2004; Wheelock and Johnson, 2003). Defects in cadherins reduce the cell-to-cell adhesion leading to metastasis in the neoplastic process (Chen et al., 2003; Naitoh et al., 1995; Nomura et al., 1995). Therefore, dysfunction and abnormal expression of the *CDH1* gene might provoke a risk to the development of human epithelial cancers (Ghadimi et al., 1999). In recent years, a number of genetic polymorphisms have been identified in the noncoding, especially, promoter region of the *CDH1* gene.

Among extensively studied polymorphisms the C to A single nucleotide polymorphism (SNP) at -160 position of the *CDH1* promoter region (*CDH1* -160C/A, rs16260) has been shown to alter the encoding and transcriptional activities of the gene (Li et al., 2000; Shin et al., 2004) leading to increased susceptibility to the onset of cervical intraepithelial neoplasia (CIN) and many other cancers (Abudukadeer et al., 2012; Rotar et al., 2016; Tan et al., 2013; Wang et al., 2008; Widschwendter et al., 2004). However, scientific literatures relating to the effect of *CDH1*-160C/A polymorphism in cervical cancer development are not only largely inconsistent but also inadequate. Moreover, we did not find any study examining the influence of *CDH1*-160C/A polymorphism in Bangladeshi female population. Thus, we designed the present case-control study with intention to evaluate the involvement of *CDH1* -160C/A polymorphism with the risk of cervical cancer development in Bangladeshi females.

Materials and Methods

Study Population

One hundred and seventeen (117) cases (cervical cancer) were enrolled in the study from National Institute of Cancer Research & Hospital (NICRH), Khulna Medical College Hospital and Islami Bank Hospital in Bangladesh. As cervical cancer is concerned only female individuals were allowed to participate in the study. Cervical cancer cases were diagnosed histologically and/or with PAP smear test in between April 2018 and June 2019. Patients were excluded from the study who have history or evidence of any other type of cancer or metastasis. We have recruited 147 (one hundred and forty-seven) randomly selected age-matched healthy females as controls from the physical examination center of the mentioned institutions and also from the neighbors during the same period. The control subjects were non-relative to the patients lacking history or evidence of cancer or any other genetic diseases. The Human Research Ethics Committee of Khulna University approved the study protocol. Written consent was taken from the participants before collecting the blood sample and the data were analyzed anonymously. During the collection of blood samples, all the participants were interviewed according to a questionnaire to collect demographic information, personal history of diseases, age at marriage and first pregnancy, family history of cervical cancer etc.

Venous blood collection and genomic DNA extraction

After selecting cases and controls, we collected around 3 mL peripheral blood samples in sterile vacuum tubes with suitable anticoagulant (EDTA-Na2; ethylenediaminetetraacetic acid disodium). Genomic DNA was extracted from collected whole blood using Daly's method with necessary modification (Daly et al., 1998). Concentration and purity of isolated DNA samples were determined by using BioDrop spectrophotometer. DNA samples showing the OD260/OD280 values between 1.8 and 2.0 were assumed as pure. The DNA samples were immediately used for genotyping or preserved at -20˚C for future use.

PCR-RFLP analysis

The collected pure DNA was then genotyped for *CDH1* -160C/A SNP by PCR–RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis as described earlier (Imtiaz et al., 2019). The primer sequences used for PCR amplification of the target polymorphic region spanning -160C/A SNP were 5'-CGCCCCGACTTGTCTCTCTAC-3' (forward) and 5'-GGCCACAGCCAATCAGCA-3' (reverse) that yielded 448 bp amplicon. Isolated genomic DNA was used as PCR template. The final volume of each PCR reaction was 25.0 µL containing 100 ng template DNA, 10X reaction buffer (2.5 µL), each primer (1.25 µL; 10 µM), Taq DNA polymerase (0.25 µL), dNTPs (0.625 µL; 2.5 mM), and nuclease-free water (q.s. to 25.0 µL). Thermal cycler was used for PCR amplification and the amplification condition was as follows: a preliminary denaturation (5 min at 95^{o}C), afterward 35 cycles of denaturation (30 sec at 94^{o}C), annealing (30 sec at 66^{o}C), extension (50 sec at 72^{o}C) in conjunction with a terminal extension for 10 min at 72^{o}C. The size of the amplified PCR products was confirmed by agarose gel electrophoresis as shown in Figure 1(A).

The amplified PCR products were then subjected to digestion using HincII restriction enzyme (Thermo Fisher Scientific, USA) following manufacturer's instruction with slight modification. In brief, 10 µl of the PCR reaction mixture (*CDH1*) was added to 10 µl of restriction enzyme mixture followed by overnight digesstion at 37^{o}C. Restriction enzyme mixture was prepared by adding 0.2 µl of HincII (2U) and 1.0 µl 10X buffer to 8.8 µl nuclease free water. The digested fragments were then separated and visualized by gel electrophoresis on ethidium bromide stained 3% agarose gel (Figure 1(B)).

Sanger sequencing

Genotyping findings from PCR-RFLP analysis were further validated by direct DNA sequencing of the PCR amplicons (448 bp) using Sanger method. From each genotyping group three samples were picked randomly. After amplification 5 µL of PCR amplicons was refined using ExoSAP-IT™ PCR Product Cleanup reagent (Thermo Fisher Scientific) and the amplicon size was confirmed by electrophoresis on 2% agarose gel. The refined PCR products were then sequenced by BigDye (v3.1) terminator sequencing kit and with 3730xl programmed sequencer (Applied Biosystems, Foster City, CA) at the Macrogen sequencing facility (Macrogen Inc.,

Seoul, Korea). Representative part of sequencing data around the SNP is shown in Figure 2. Sequencing was carried out using the same reverse primers.

Equipment and settings

The UVP Bio Imaging System (Cambridge, UK) was used for gel imaging of stained gels. The exclusive software controlled the camera functions, live preview of images, image capture and image enhancement. The gel image was saved as JPEG format. The original image was cropped and slightly processed (changing brightness and contrast) using Microsoft Office software packages (Photos). The sequencing data was generated with 3730xl programmed sequencer (Applied Biosystems, Foster City, CA) at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea). The sequencing data was obtained in a number of formats including Microsoft Edge PDF Document (.pdf) format.

Statistical Analysis

IBM SPSS Statistics (v25.0) software package was used for many of the statistical analysis. To authenticate DNA genotyping frequencies, Hardy-Weinberg equilibrium testing was performed out employing the $\chi 2$ test. The $\chi 2$ test (using two-sided contingency table) was carried out to calculate genotypic distribution and allelic frequencies of *CDH1* -160C/A. Multiple logistic regression model analysis was used to determine odds ratio (OR) and 95% confidence interval (CI) and p-values less than 0.05 were deemed as statistically significant.

Results

Characteristics of the study population

The study included 117 cancer cases and 147 healthy controls where the average age of cases and controls were 44.1±11.1 (range 21-71) and 44.5±14.1 (range 18-75) year, respectively. Table 1 summarizes the distribution of

Table 1. Distribution of Selected Demographic and Pathophysiologic Variables among the Study Population

Demographic Variables	Cases (n = 117) n or (%)	Controls (n = 147) n or (%)
Age (years)		
Average	44.13±11.12	44.46±14.04
Range	21-71	18-75
BMI categories (kg/m^2)		
Under and normal weight (<25)	101 (86.32)	102 (69.39)
Overweight and obesity (≥25)	16 (13.68)	45 (30.61)
Diabetic status		
Diabetics	60 (51.28)	25 (17.00)
Non-diabetics	57 (48.72)	122 (83.00)
Family history		
Yes	69 (58.98)	
No	48 (41.02)	
Age at diagnosis (Year)		
≤35	32 (27.35)	
36-50	62 (52.99)	
>50	23 (19.66)	
Age at Marriage (Year)		
Early Marriage (<18)	93 (79.49)	
Legal Marriage (≥18)	24 (20.51)	
Age at First Pregnancy (Year)		
<19	89 (76.06)	
≥19	28 (23.93)	
Husband's Smoking Status		
Smoker	79 (67.52)	
Non-Smoker	38 (42.48)	
Stage of Cancer		
Stage I	28 (23.93)	
Stage II	67 (57.26)	
Stage III	22 (18.80)	

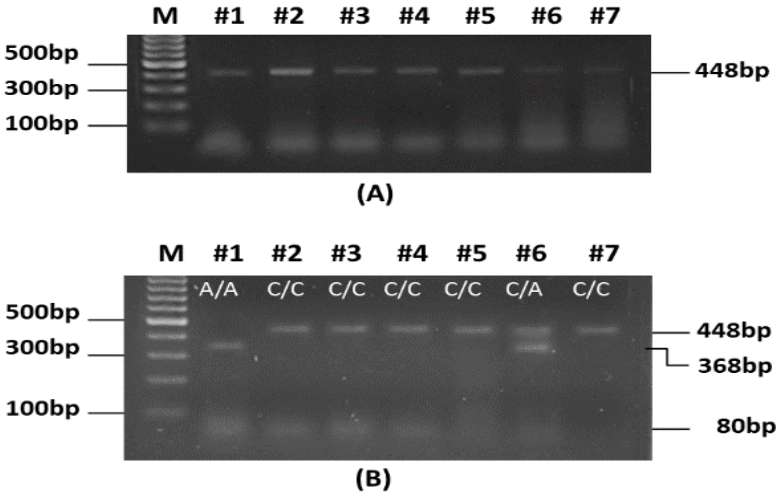

Figure 1. Visualization of *E-cadherin (CDH1)* -160C/A Polymorphism. (A) PCR amplified products (448 bp) of E-cadherin on 2% agarose gel. Lane 1-7, PCR products & Lane M, 100bp DNA ladder (B) Restriction enzyme (HincII) digestion fragments of E-cadherin subjected to electrophoresis on 3% agarose gel. Lane M, 100bp DNA ladder. Lane 2, 3, 4, 5 and 7, homozygote (C/C; 448 bp); Lane 6, heterozygote (C/A; 448, 368, 80 bp) & Lane 1, variant homozygote (A/A; 368, 80 bp). The gel images have been cropped from the original images. The uncropped images are provided as supplementary Figure 1 and included in supplementary files.

Table 2. Genotype Frequencies of *CDH1* Polymorphism in Relation to Cervical Cancer Cases and Controls

Gene	Genotype	Cases, n (%) (n=117)	Control, n (%) (n=147)	Odds ratio	95 % CI	p- value
CDH1-160C/A	C/C	75 (64.10)	114 (77.55)	1	-	-
	C/A	32 (27.35)	29 (19.73)	1.677	0.938 to 2.998	0.081
	A/A	10 (8.55)	4 (2.72)	3.800	1.150 to 12.561	0.029
	C/A+A/A ('any A')	42 (35.90)	33 (22.44)	1.934	1.126 to 3.323	0.017
	Allele					
	C	182 (77.78)	257 (87.41)	1	-	-
	A	52 (22.22)	37 (12.59)	1.985	1.250 to 3.151	0.004

C/C, normal homozygote; C/A, heterozygote; A/A, variant homozygote; OR, Odds ratio; CI, Confidence interval

the chosen demographic and pathophysiologic variables.

Genotypic and allelic distribution of CDH1 -160C/A (rs16260) polymorphism in cervical cancer

The -160C/A single nucleotide polymorphism of *CDH1* gene was successfully genotyped using PCR-RFLP method as described earlier. However, PCR-RFLP findings were further validated by direct DNA sequencing of some representative samples. Figure 2 shows part of sequencing data around the target *CDH1* -160C/A SNP. Genotyping frequencies in controls were assessed for Hardy–Weinberg equilibrium (HWE) and the distribution of *CDH1* -160C/A genotypes did not deviate significantly from HWE (p>0.05). The genotypic distribution and allelic frequencies in both cervical cancer patients and healthy controls together with their involvement with cervical cancer risk are demonstrated in Table 2. The homozygous -160C/C genotype was most frequently demonstrated in both cases and controls (64.10% and 77.55%, respectively) and hence, was taken as normal genotype as well as reference to compare with the other genotypes. The resultant frequencies of normal homozygous (C/C), heterozygous (C/A) and variant homozygous (A/A) genotypes were 64.10, 27.35 and 8.55% in cases, and 77.55, 19.73 and 2.72% in controls, respectively.

Both variant homozygous (A/A) and combined (C/A+A/A) or 'any A' genotype frequencies were significantly increased in cervical cancer cases (8.55% vs 2.72% and 35.90% vs 22.44%, respectively), and presented significant association exhibiting 3.80-fold (odds ratio, OR=3.80, 95% confidence interval, CI=1.150 to 12.561, p=0.029) and 1.93-fold (OR = 1.93, 95% CI = 1.126 to 3.323, p=0.017) increase in cervical cancer risk, respectively. Although the frequency of the variant heterozygous (C/A) genotype was found higher in cases compared to controls (27.35% vs 19.73%), the difference in results was not statistically significant.

The frequencies of -160C and -160A allele were 77.78 and 22.22% in cases, whereas 87.41 and 12.59% in controls, respectively (Table 2). Statistical analysis suggested variant -160A allele to be positively correlated with enhanced cervical cancer risk (OR=1.985, 95% CI=1.250 to 3.151, p=0.004).

Association between CDH1 -160C/A polymorphism and clinicopathological variables in cervical cancer patients

The genotypic distribution of *CDH1* -160C/A SNP was compared with the demographic and clinicopathological characteristics of cervical cancer patients like age, BMI, diabetes status, family history, age at marriage and first pregnancy, passive smoking status (smoking husband)

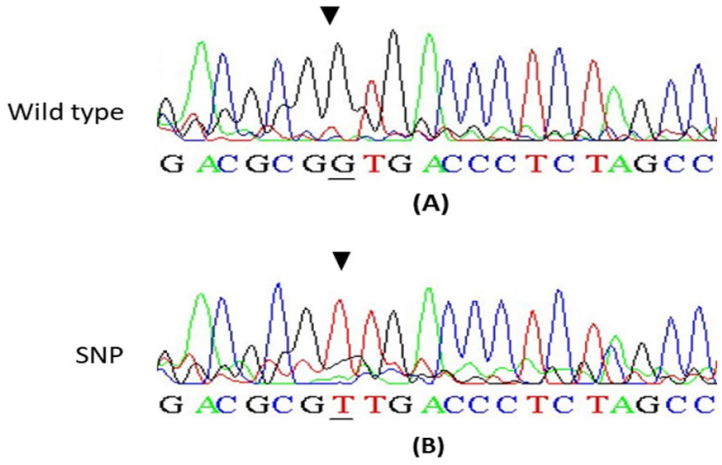

Figure 2. The Chromatograms of the *CDH1* -160C/A SNP by Direct Sequencing Using Reverse Primer. (A) Normal homozygote (G/G - complementary of C/C). (B) Variant homozygote (T/T - complementary of A/A). Arrows indicate the SNP positions. Representative part of sequencing data around the SNP is cropped from the original image. The uncropped image file provided as supplementary Figure 2 and included in supplementary files.

Table 3. Clinicopathological Relevance of *CDH1*-160C/A Polymorphism in Cervical Cancer Patients

Variables	Cases, n=117				p- values
	No. of patients	C/C n, (%)	C/A n, (%)	A/A n, (%)	
Age at Diagnosis (year)					
≤35	32 (27.35)	19 (59.38)	11 (34.38)	2 (6.25)	0.629
36 - 50	62 (52.99)	39 (62.9)	16 (25.81)	7 (11.29)	
>50	23 (19.66)	17 (73.91)	5 (21.74)	1 (4.35)	
BMI Categories (kg/m2)					
Normal weight (<25)	101 (86.32)	66 (65.34)	27 (26.73)	8 (7.92)	0.733
Over Weight/Obese (≥25)	16 (13.68)	9 (56.25)	5 (31.25)	2 (12.5)	
Diabetes Status					
Diabetics	60 (51.28)	39 (65)	16 (26.67)	5 (8.33)	0.979
Non-Diabetics	57 (48.72)	36 (63.16)	16 (28.07)	5 (8.78)	
Family History					
Yes	69 (58.98)	40 (57.97)	23 (33.33)	6 (8.69)	0.203
No	48 (41.02)	35 (72.92)	9 (18.75)	4 (8.33)	
Age at Marriage (Year)					
Early Marriage (<18)	93 (79.49)	60 (64.52)	28 (30.12)	5 (5.38)	0.037
Legal Marriage (>18)	24 (20.51)	15 (62.5)	4 (16.67)	5 (20.83)	
Age at First Pregnancy (Year)					
<19	89 (76.06)	58 (65.17)	26 (29.21)	5 (5.62)	0. 118
≥19	28 (23.93)	17 (60.71)	6 (21.42)	5 (17.86)	
Husband Smoking Status					
Smoker	79 (67.52)	49 (62.02)	23 (29.11)	7 (8.86)	0.791
Non-Smoker	38 (42.48)	26 (68.42)	9 (23.68)	3 (7.89)	
Stage of Cancer					
Stage I	28 (23.93)	19 (67.86)	8 (28.57)	1 (3.57)	0.247
Stage II	67 (57.26)	41 (61.19)	21 (31.34)	5 (7.46)	
Stage III	22 (18.80)	15 (68.18)	3 (13.64)	4 (18.18)	

and cancer stages etc (Table 3). We observed that C/A genotypes were significantly higher in patients with early marriage (before 18 years) history compared to the non-early marriage group. We found *CDH1* -160C/A SNP to be significantly associated with early marital history (before 18 years). Patients with a history of early marriage were more susceptible to cervical cancer development compared to patients with legal marital age ($\chi^2 = 6.605$, p=0.037) (Table 3). However, we did not find significant association with any other studied pathophysiologic variables.

Discussion

In Bangladesh, according to recent report, cervical cancer emerges as the second most common cancer in females aging 15 to 44 years (Bruni et al., 2019). To the best of our knowledge, this is the first study to report the relationship between *CDH1* -160C/A polymorphism and cervical cancer incidence among Bangladeshi females. Our results suggest that homozygous -160A/A genotypes and combined (C/A+A/A) or 'any A' genotypes present an enhanced risk of cervical cancer development.

CDH1, a tumor suppressor gene, exerts its crucial role by taking part in cell adhesion, diversity, cellular polarity and in tissue morphology (Grunwald, 1993). Loss of functions of the gene because of genetic polymorphism or DNA methylation has been attributed to be an indicator of tumor progression (Abudukadeer et al., 2012; Ghadimi et al., 1999). The promoter area of the *CDH1*gene was reported to be greatly polymorphic in some ethnicities (Nakamura et al., 2002). Our candidate *CDH1* -160C/A (rs16260) SNP is located in the promoter region near the transcription start site (TSS) (Jonsson et al., 2004). This polymorphism has been reported to be associated with the prevalence of a number of cancers e.g., gastric, pancreatic, prostate, urothelial, colorectal cancer etc (Cattaneo et al., 2006; Imtiaz et al., 2019; Li et al., 2000; Shin et al., 2004; Tran et al., 2004; Wang et al., 2012).

However, findings regarding the impact of *CDH1* -160C/A polymorphism on different cancers other than cervical cancer remains inconsistent and even some findings were contradictory for the same cancers from different ethnicities. In a case-control study, Lei (2002) did not find any association between the selected *CDH1* -160C/A polymorphism and breast cancer (Lei et al., 2002). Conversely, Tipirisetti (2013) showed -160A allele to be positively associated with advanced stage breast

cancer in South Indian women (Tipirisetti et al., 2013). In addition, a Taiwanese study reported that the presence of -160A allele was significantly correlated with advanced clinical stages of liver cancer (Chien et al., 2011). In contrast, a research study conducted in pancreatic cancer revealed that significantly reduces the risk of lymph node metastasis and clinical stages (Zhao et al., 2015).

In our present study, we report that variant homozygous genotype (-160A/A) is strongly associated with cervical cancer risk that depicts 3.80-fold increased threat for developing the malignancy. In addition, combined heterozygous plus variant homozygous (C/A+A/A) or 'any A' genotype also demonstrated positive association with 1.93-times higher susceptibility to cervical cancer development. Then we assessed the allelic distributions of *CDH1* -160C/A polymorphism in the same population where the occurrence of -160A allele was greater in cases conferring 1.98-fold risk elevation compared to -160C allele.

The number of studies relating *CDH1* -160C/A polymorphism to cervical cancer threat is extremely limited. Kang et al (2008) investigated the impact of *CDH1* -160C/A SNP on cervical intraepithelial neoplasm (CIN) and cervical cancer in Korean residents but found no significant association (Kang et al., 2008). Rotar (2016) performed a case-control study enrolling 70 cases and 107 age-matched healthy controls and reported a significant association of -160A variant with high-grade squamous intraepithelial lesion (HSIL) and carcinoma in situ (CIS) (Rotar et al., 2016). These findings are similar with our findings where we show the variant -160A allele to be associated with increased cervical cancer risk. However, we did not get any statistically significant correlation between heterozygosity (-160C/A) and the cancer (p= 0.081) in our current study population. We think this is because of a limited number of samples in our study population.

Different research groups have attempted to unveil the influence of *CDH1* -160C/A polymorphism. In an in vitro study the -160A allele was found to reduce its transcriptional activity by 68% when compared to -160C allele supporting the fact that the transcriptional activity of *CDH1* gene is directly altered by -160C/A polymorphism (Li et al., 2000). Akbas et al., (2013) have also shown that transition from C to A at position -160 imparted diminished transcriptional action, which ultimately resulted in decreased *CDH1* expression. And finally, it is hypothesized that the variant -160A allele induced reduced *CDH1* expression might be responsible for increased susceptibility to different cancers (Humar et al., 2002; Tsukino et al., 2003; Verhage et al., 2002; Zhang et al., 2003).

Later, we investigated the correlation of *CDH1* -160 genotypes with some demographic and clinicopathological parameters such as age, BMI, diabetes status, family history, age at marriage, age at first pregnancy, passive smoking status (husband's smoking status) and stages of cancer within the cervical cancer patient group. From the comparison, our data shows that age at marriage are more likely to be associated with cervical cancer risk and marriage at earlier age (before 18 years old) imparts about 6.60-fold higher risk compared to marriage at later age (after 18 years old) in our study population. Our finding resembles to some other studies who disclosed a significant correlation between early marriage and the threat to cervical cancer occurrence (Chaouki et al., 1998; Christofherson and Parker, 1965; Nour, 2006). Although a number of studies confirmed a significant correlation between incidence of cervical cancer and passive smoking status (ever smoking husband), (Brown et al., 1982; Louie et al., 2011; Tokudome, 1997) our study did not find this correlation in our study population. This might result from a limited number of samples in our study group. Extended study with a bigger sample size is crucial to validate the outcome.

Relatively smaller sample size was an important limitation for which the study lacked sufficient strength to reveal association between genotypes and disease, as well as gene and environment interactions. Large population-based further studies in different ethnicities are necessary to illuminate the outcomes. Studies relating to gene expression are also necessary to understand more clearly how the putative SNPs influence the cervical cancer development and its prognosis.

In sum, we report that variant homozygous (A/A) and combined (C/A+A/A) genotypes of *CDH1*-160C/A polymorphism may impart an enhanced threat to the development of cervical cancer among Bangladeshi women. In addition, women carrying these genotypes along with marriage at earlier age are more vulnerable.

Author Contribution Statement

Md. Abdur Rahman conceptualized the presented idea, carried out lab experiments and wrote original draft. Md. Mehedi Hasan: Contributed to blood sample and data collection, and experimental design and method validation. Amir Hossain: Contributed to statistical analysis, editing and formatting the draft manuscript. Khan Monjurul Alam: Blood samples and data collection, DNA extraction. Razia Sultana: Verified analytical method, experimental results and contributed to statistical analysis. Md. Abdul Mazid: Contributed to data interpretation, writing-review and editing especially language checks. Md. Mustafizur Rahman*: Developed the idea, administrated the project, supervised the investigation and findings of the work. All authors discussed the results, contributed to the final manuscript and approved the final version (*, corresponding author).

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General

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Approval

The study is part of an approved Masters' thesis of a student.

Ethical approval

The Human Research Ethics Committee of Khulna University approved the study protocol, and the study was conducted according to the Helsinki declaration and its subsequent revisions. Informed consent was obtained from all participants.

Data availability

Data generated during this study are included in this published article. The DNA sequencing data have been deposited in DDBJ database under accession number DRR410073, which can be accessed through the following link: https://ddbj.nig.ac.jp/resource/sra-run/DRR410073. However, some data e.g., individual pathophysiological data and individual genotyping findings that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest disclosures

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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