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Polymorphisms of the interleukin-6 (*IL-6*) gene contribute to cervical cancer susceptibility in Bangladeshi women: A case-control study

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Abstract

Background and Aims: Cervical cancer is characterized by abnormal cell growth in the lining of cervix and it is the second major cause of cancer-related deaths among females in Bangladesh. Interleukin-6 (*IL-6*) is a multifunctional cytokine that has been heavily linked with cervical cancer. Our aim was to investigate the association of two promoter single-nucleotide polymorphisms (SNPs) of *IL-6* (rs1800795 and rs1800797) with the susceptibility of cervical cancer in Bangladeshi women.

Methods: DNA was extracted from venous blood samples from cervical cancer patients ($n = 126$) and healthy controls ($n = 120$). Polymerase chain reaction-restriction fragment length polymorphism was used for genotyping of the selected SNPs. Logistic regression was performed to calculate the odds ratio (OR) with 95% confidence interval (CI) and p values.

Results: We found a significant association between rs1800795 and rs1800797 polymorphisms and cervical cancer. For, rs1800795 (G > C) the GC heterozygous genotype (OR = 2.80, 95% CI = 1.55–5.07, $p = 0.0007$) and CC mutant homozygous genotype (OR = 3.5, 95% CI = 1.29–9.51, $p = 0.014$) conferred an increased risk of cervical cancer. In case of rs1800797 (G > A) polymorphism, the AG heterozygous genotype (OR = 6.94, 95% CI = 3.76–12.81, $p < 0.0001$) and AA mutant homozygous genotype (OR = 3.88, 95% CI = 1.12–13.51, $p = 0.0332$) also exhibited an elevated risk of cervical cancer. Use of contraceptives was found as risk factor and patients who smoke were carriers of both the risk alleles and thus had an increased risk of cervical cancer.

Conclusion: Our findings suggest that polymorphism of rs1800795 and rs1800797 of the *IL-6* gene play a significant role in cervical cancer susceptibility in Bangladeshi women.

KEYWORDS

Bangladesh, cervical cancer, interleukin-6, polymorphism, rs1800795, rs1800797

Monishita Shaswati and Fihima Hossain Oeishy contributed equally to this study.

1 | INTRODUCTION

Cervical cancer is a major prevalent malignancy among women. In 2020, it was reported as the fourth leading cause of cancer death among females worldwide, which accounts for 6.5% of newly diagnosed cancer cases and 7.7% of cancer-related deaths. There were 604,127 new cases and 341,831 deaths from cervical cancer, most of which occurred in low- and middle-income countries.[1] In Bangladesh, cervical cancer ranks second for both the most frequently diagnosed malignancy and cancer-related deaths.[2] Persistent infection from human papillomavirus (HPV) is considered as the main causative agent of cervical cancer.[3] However, HPV alone is insufficient to induce tumor growth, and host immune response and genetic factors play a critical role in the malignant transformation of the cervical epithelium.[4]

Chronic inflammation, regulated by immune cells, strongly correlates with tumor growth[5] Studies have shown that immune cell mediators, such as cytokines, are essential modulators in viral replication.[6] Therefore, mutations in genes related to immunity can affect the immune response and influence the development of cervical cancer. Interleukin-6 (*IL-6*) is a pleiotropic cytokine that plays an important role in regulation of immune response, cell proliferation, hematopoiesis, and inflammation.[7] It induces vascular endothelial growth factor gene expression and favors tumor growth.[8] Elevated levels of *IL-6* have been found in tumor tissues[9–11] and several single-nucleotide polymorphisms (SNPs) in the *IL-6* gene have been implicated with increased risk of cervical cancer.[6,12–14]

Two promoter polymorphisms, rs1800795 (−174G/C) and rs1800797 (−597A/G), the *IL-6* gene have been associated with a wide variety of malignancies,[15,16] including cervical cancer.[14,17,18] These two SNPs are located in the 5′ flanking region (7p15.3) of the *IL-6* gene promoter.[17,19] Polymorphisms in this promoter region can affect the transcription rate and plasma levels of *IL-6*.[20] Moreover, *IL-6* −174G>C (rs1800795) has been associated with a negative regulatory effect on reporter gene expression.[21] A single nucleotide change from G to C at position −174 is thought to influence the binding of the glucocorticoid receptor, which results in the suppression of *IL-6* transcriptional activity.[22] The *IL-6* expression in tumor cells is associated with auto and paracrine stimulation of tumor cell proliferation.[23] It also activates several signaling pathways, leading to upregulation of antiapoptotic proteins and induction of proangiogenic cytokines.[24]

A recent meta-analysis found that rs1800795 polymorphism was significantly associated with an increased risk of cervical cancer in Asian (Korean, Chinese, and Indian) women.[25] Several studies have reported that the presence of at least one C allele in the *IL-6* promoter region (−174G>C) increased the risk of cervical cancer in Eastern Chinese[26] and Indian populations.[27] In a different meta-analysis, the C allele of rs1800795 polymorphism was similarly linked to an increased risk of cervical cancer in women of Han Chinese, Brazilian, Indian, and Caucasian ethnicities.[14] However, negative association of this polymorphism with cervical cancer has also been reported in Caucasian and Brazilian women.[25,28]

Several studies reported that the presence of *IL-6* rs1800797 (−597A/G) polymorphism conferred an increased risk of cervical cancer in population from different ethnicities. Studies conducted in Han Chinese and Indian population reported a significant association between this polymorphism and cervical cancer.[29,30] Gupta et al. have reported that −597G allele of *IL-6* rs1800797 increased the risk of cervical cancer by up to 6.2 times ($p < 0.001$) in the North Indian study population.[31] However, negative association between this SNP and cervical cancer has also been reported in Swedish and Lithuanian women of Caucasian ancestry.[32,33] Overall, the association between these two putative SNPs of *IL-6* and cervical cancer is inconsistent.

Therefore, the aim of our study was to evaluate the association between two *IL-6* polymorphisms rs1800795 and rs1800797 and cervical cancer in Bangladeshi women. We also investigated the correlation of the SNPs with clinicopathological characteristics.

2 | MATERIALS AND METHODS

2.1 | Selection of study population

One hundred twenty-six cervical cancer patients and 120 age-matched healthy controls (18–65 years) were recruited in this study. Cervical cancer patients were recruited from Dhaka Cancer and General Hospital Dhaka, and Dhaka Medical College and Hospital in Dhaka, Bangladesh. Using the G*Power software, the required sample size was estimated a priori with an effect size of $d = 0.8$ (large effect) and $\alpha = 0.05$, which resulted in a total required sample size of 246. After histopathological diagnosis, the patients were staged in accordance with the International Federation of Gynecology and Obstetrics staging standards.[34] Previous medical records were checked and all the patients were non-HPV infected and practiced abstinence from alcohol throughout their entire lives. After undergoing a complete medical examination, age-matched healthy controls were recruited, and healthy controls with a history of mental illness, head injury, trauma, pregnancy, substance abuse, or alcohol consumption were excluded from the study. All study participants signed written consent forms and the study was carried out in accordance with the Declaration of Helsinki and its subsequent amendments.[35] The ethical review committee of Dhaka Medical College and Hospital approved the study protocol (ERC-DMC/2020/151).

2.2 | DNA extraction and genotyping

About 5 mL venous blood was collected from each study participant in potassium EDTA tubes (BD Vacutainer® blood collection tubes, Becton and Dickinson and Company). They were stored at −80°C until further experiments. DNA extraction was performed using Bioneer® genomic DNA extraction kit (Bioneer Corporation) using the manufacturer's protocol. The quality of

TABLE 1 Primers for *IL-6* gene polymorphisms rs1800795 and rs1800797.

Target SNP	Primer Sequence	PCR product (bp)	Restriction enzyme	PCR products after digestion (bp)
IL-6 174G>C (rs1800795)	F 5′-TGACTTCAGCTTTACTCTTGT-3′ R 5′-CTGATTGGACTTATTAAG-3′	198	*Hin1II*	GG = 167, 31 GC = 167, 122, 45, 31 CC = 122, 45, 31
IL-6 597G>A (rs1800797)	F 5′-GGAGTCACACACTCCACCT-3′ R 5′-CTGATTGGAAACCTTATTAAG-3′	525	*FokI*	GG = 468 AG = 525, 468 AA = 525

Abbreviations: PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

TABLE 2 Distribution of demographic characteristics of cervical cancer patients and controls.

Characteristics	Cases (n = 126) (%)	Controls (n = 120) (%)	χ^2 (p value)
Age (years)			
≤45	58 (46.0)	67 (55.8)	2.37 (0.124)
>45	68 (54.0)	53 (44.2)	
Dwelling			
Urban	26 (20.6)	21 (17.5)	0.39 (0.627)
Rural	100 (79.4)	99 (82.5)	
Menstrual status			
Pre-menopause	72 (57.1)	59 (49.2)	1.57 (0.250)
Post-menopause	54 (42.9)	61 (50.8)	
Tobacco use			
Smokers	33 (26.19)	19 (15.83)	3.96 (0.05)
Non-smokers	93 (73.81)	101 (84.17)	
Parity			
0–7	122 (96.8)	115 (95.8)	0.17 (0.744)
>7	4 (3.2)	5 (4.2)	
Contraception			
Oral pills	54 (42.9)	67 (55.8)	1.03 (0.311)
Others[a]	15 (11.9)	3 (2.5)	6.15 **(0.013*)**
Combination[b]	10 (7.9)	6 (5.0)	0.65 (0.422)
None	47 (37.3)	44 (36.7)	–
Family history of cancer (first-degree relatives)			
Yes	20 (15.88)	24 (20)	0.71 (0.399)
No	106 (84.12)	96 (80)	
Stage of cancer			
IA–IB	24 (19.05)		
IIA–IIB	67 (53.18)		
IIIA–IIIB	35 (27.78)		
Histopathology			
Squamous cell carcinoma	103 (81.75)		
Adenocarcinoma	17 (13.50)		
Others	6 (4.75)		
Tumor grade			
I	11 (8.73)		
II	99 (78.58)		
III	16 (12.70)		

[a]Others: Intrauterine device (IUD) + Barrier (cervical cap, diaphragm, and female condom).

[b]Combination: Oral pills + condom(male), Oral pills + combined injectable contraceptives (CIC).

*$p < 0.05$.

DNA was examined using a NanoDrop spectrophotometer (Thermo Fisher Scientific). The selected SNPs were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the previously described method.[36,37] The PCR products of *IL-6* 174G>C (166 bp) and *IL-6* 597A>G (525 bp) were digested for 16 h with restriction enzymes *Hin1*II and *Fok*I, respectively. Then the amplicons were visualized on 2% agarose gel using ethidium bromide by gel electrophoresis. Table 1 contains the details of the PCR protocol and Supporting Information contain RFLP images for both SNPs.

2.3 | Statistical analysis

All statistical analyses were performed using two-tailed tests. The chi-square test was used to compare the categorical variables, while *t* test was used to compare the continuous variables in demographic data, genotype frequencies, and clinicopathological data. The Hardy–Weinberg Equilibrium was calculated using chi-square test to measure the deviation in the genotype frequencies in the healthy control group from the patients' group. Multinomial logistic regression was used to calculate the adjusted odds ratio controlling for relevant covariates (age, menstrual status, tobacco use) along with 95% confidence intervals (CIs) and p values. $p < 0.05$ was considered statistically significant. The statistical analyses were performed using SPSS version 23 (SPSS, Inc.).

TABLE 3 Genotype frequencies of *IL-6* gene polymorphisms in cervical cancer patients and controls.

	Genotypes	Cases	Controls	Adjusted odds ratio	95% CI	*p* Value
rs1800795		*n* = 126 (%)	*n* = 120 (%)			
	GG	65 (51.6)	91 (75.8)	Ref.	–	–
	GC	46 (36.5)	23 (19.2)	2.80	1.55–5.07	**0.0007**
	CC	15 (11.9)	6 (5.0)	3.50	1.29–9.50	**0.0140**
	GC + CC	61 (48.4)	29 (24.2)	2.95	1.71–5.08	**0.0001**
	C Allele	76 (60.3)	35 (29.2)	2.53	1.61–3.96	**<0.0001**
rs1800797						
	GG	50 (39.7)	97 (80.9)	Ref.	–	–
	AG	68 (53.9)	19 (15.9)	6.94	3.76–12.81	**<0.0001**
	AA	8 (6.4)	4 (3.2)	3.88	1.12–13.51	**0.0332**
	AG + AA	76 (60.3)	23 (19.2)	6.41	3.60–11.43	**<0.0001**
	A Allele	84 (66.7)	27 (22.5)	3.23	2.01–5.20	**<0.0001**

Note: Statistically significant values are made bold.
Abbreviation: CI, confidence interval.

3 | RESULTS

3.1 | Characteristics of the study population

Table 2 describes the demographic and clinicopathological characteristics of the participants. A total of 246 women were selected for this study comprising 126 cervical cancer patients and 120 healthy controls. A marginally significant difference ($p = 0.047$) was observed between the cases and controls regarding tobacco use as there were more smokers (26.19%) in the case group compared to the healthy control group (15.83%). This is in agreement with previous studies which demonstrated smoking as a risk factor for cervical cancer.[38,39] We also found a significant difference ($p = 0.013$) regarding the use of contraceptives as women who used intrauterine device, cervical cap, barrier, and female condom were more prone to the risk of cancer. This is similar to our findings with a different cohort of cervical cancer patients, where we found that similar contraceptives increase the risk of cervical cancer in Bangladeshi women.[40] However, no statistically significant difference was observed for age, menopausal status, dwelling status, parity, postmenopausal status, number of children, and family history of cancer.

3.2 | Analysis of genotype frequencies of rs1800795 and rs1800797 of IL-6 gene

The distribution of genotype frequencies for both SNPs in cervical cancer patients and healthy controls is summarized in Table 3. For *IL-6* rs1800795 polymorphism, cervical cancer patients had higher frequencies of GC heterozygous genotype compared to controls (36.5% vs. 19.2%). Logistic regression analysis showed 2.80-fold more risk of developing cervical cancer in carriers of GC genotype (adjusted OR = 2.80, 95% CI = 1.55–5.067, $p = 0.0007$). Similarly, the prevalence of CC mutant homozygous genotype was higher in cases than in controls (11.9% vs. 5%), showing a statistically significant relationship with cervical cancer development (adjusted OR = 3.5, 95% CI = 1.29–9.51, $p = 0.014$). The presence of the C allele conferred 2.5 times more risk of cervical cancer (adjusted OR = 2.53, 95% CI = 1.62-3.96, $p < 0.0001$).

Frequencies of AG heterozygous and AA mutant homozygous genotypes of *IL-6* rs1800797 polymorphism were higher in cases compared to controls (53.9% vs. 15.9% and 6.4% vs. 3.2%, respectively). The presence of AA genotype was significantly associated with cervical cancer, increasing risk by 3.88 times (adjusted OR = 3.88, 95% CI = 1.12–13.52, $p = 0.0332$). AG genotype conferred even greater risk (6.943 times) of developing cervical cancer (adjusted OR = 6.95, 95% CI = 3.76–12.81, $p \leq 0.0001$). The dominant model (GG vs. AG + AA) also showed a significant association (adjusted OR = 6.41, 95% CI = 3.60–11.43, $p < 0.0001$).

3.3 | Association of IL-6 polymorphisms with clinicopathological characteristics

Analysis of rs1800795 with different clinicopathological characteristics of the patients showed that smokers were carriers of the risk allele C at much higher frequency compared to non-smokers ($p = 0.044$; Table 4). Similar results were also found for rs1800797, where smokers were carriers of the risk allele A in higher frequency compared to non-smokers ($p = 0.039$; Table 5). Moreover, we found that for rs1800797, family history of cancer was a contributing factor. Patients with first-degree relatives with cancer were more frequent carriers of the mutated allele A compared to patients with no family history of cancer.

TABLE 4 Correlation of rs1800795 polymorphisms with clinicopathological characteristics of the patients.

Characteristics	rs1800795 carrier *n* = 61	rs1800795 noncarrier *n* = 65	OR (95% CI)	*p* Value
Age (years)				
≤45	32	26	Ref.	1.000
>45	29	39	0.61 (0.30–1.23)	0.162
Dwelling status				
Rural	45	55	Ref.	1.000
Urban	16	10	1.96 (0.81–4.43)	0.137
Menstrual status				
Post-menopause	30	24	Ref.	1.000
Pre-menopause	31	41	0.61 (0.30–1.23)	0.166
Tobacco use				
Non-smokers	40	53	Ref.	1.000
Smokers	21	12	2.32 (1.02–5.26)	**0.044***
Parity				
0–7	58	64	Ref.	1.000
>7	3	1	3.31 (0.34–32.72)	0.306
Contraception				
None	21	26	Ref.	1.000
Oral pills	28	26	1.33 (0.61–2.92)	0.472
Others[a]	7	8	1.08 (0.34–3.48)	0.893
Combination[b]	5	5	1.24 (0.32–4.86)	0.759
Family history of cancer				
No	49	57	Ref.	1.000
Yes	12	8	1.75 (0.66–4.62)	0.262

[a]Others: Barrier (cervical cap, diaphragm, female condom) + intrauterine device (IUD).
[b]Combination: Oral pills + condom (male), Oral pills + combined injectable contraceptives (CIC).
*$p < 0.05$.
Abbreviations: CI, confidence interval; OR, odds ratio.

4 | DISCUSSION

The aim of our study was to explore the association between two *IL-6* gene polymorphisms and the risk of cervical cancer in Bangladeshi women. We investigated two promoter region SNPs rs1800795 and rs1800797 in cervical cancer patients and controls of Bangladeshi ethnicity. These SNPs have the potential to be used as a biomarker for cervical cancer and may act as expression quantitative trait loci for gene and protein expression in cancer tissues. Our analysis showed that polymorphism in both the rs1800795 and rs1800797 SNPs confer an increased risk of developing cervical cancer. Smoking and use of contraception were also found to be significant factors for cervical cancer.

For the SNP rs1800795, we found that the GC and CC genotypes increase the risk of cervical cancer by 2.825 and 3.002 times, respectively. Our finding is in agreement with several previous genetic epidemiological studies where GC and CC genotypes and C allele of the rs1800795 SNP was found to have a significant association with cervical cancer in Indian,[41] Lithuanian,[33] and Tunisian women.[18] This finding is further strengthened by multiple meta-analysis reports which demonstrated a positive association between the G > C mutation of rs1800795 with cervical cancer.[14,25,42,43] Moreover, a recent meta-analysis by Harun-Or-Roshid et al. found that the presence of C allele increases the risk of cervical cancer in women of Asian and African ancestry.[15]

On the other hand, no significant association was found between rs1800795 polymorphism and cervical intraepithelial neoplasia in the Austrian population of Caucasian ancestry.[28] Additionally, Zidi et al. demonstrated that the G allele and GG genotype might have a protective effect against the development of cervical cancer.[18] Overall, the majority of genetic epidemiological studies and meta-analyses suggest polymorphism at rs1800795 of *IL-6* gene as a prime

TABLE 5 Correlation of rs1800797 polymorphisms with clinicopathological characteristics of the patients.

Characteristics	rs1800797 carrier *n* = 76	rs1800797 noncarrier *n* = 50	OR (95% CI)	*p* Value
Age (years)				
≤45	31	27	Ref.	1.000
>45	45	23	1.71 (0.83–3.50)	0.147
Dwelling status				
Rural	61	39	Ref.	1.000
Urban	15	11	0.87 (0.37–2.09)	0.759
Menstrual status				
Pre-menopause	43	29	Ref.	1.000
Post-menopause	33	21	1.06 (0.52–2.18)	0.875
Tobacco use				
Non-smokers	51	42	Ref.	1.000
Smokers	25	8	2.58 (1.05–6.30)	**0.039***
Parity				
0–7	74	48	Ref.	1.000
>7	2	2	0.65 (0.09–4.76)	0.670
Contraception				
None	29	18	Ref.	1.000
Oral pills	33	21	0.98 (0.44–2.18)	0.952
Others[a]	7	8	0.54 (0.17–1.76)	0.308
Combination[b]	7	3	1.45 (0.33–6.33)	0.623
Family history of cancer				
No	58	48	Ref.	1.000
Yes	16	4	3.31 (1.04–10.57)	**0.043***

[a]Others: Barrier (cervical cap, diaphragm, female condom) + Intrauterine device (IUD).
[b]Combination: Oral pills+ condom (male), Oral pills+ combined injectable contraceptives (CIC).
*$p < 0.05$.
Abbreviations: CI, confidence interval; OR, odds ratio.

factor for cervical cancer. But studies need to be done in larger population with larger sample size and different ethnic background to confirm the results.

Regarding the SNP rs1800797, we found a significant association with cervical cancer. Compared to GG genotype, AG and AA genotypes confer 6.943 and 3.88 times more risk of developing cervical cancer. Our finding is similar to a previous study conducted in Bangladeshi population which found that the presence of AG and AA genotypes increases the risk of cervical cancer.[29] Moreover, rs1800797 polymorphism was reported to be a risk factor for cervical cancer in Chinese and North Indian populations.[30,31] In contrast, a lack of association was observed between polymorphism at this SNP and cervical cancer in the Swedish and Lithuanian population of Caucasian ethnicity.[32,33] The differences in results may be due to differences in ethnic background and genotypic variations may alter the expression of *IL-6* differently in women of diverse ethnicity and may act as protective or risk factor for cervical cancer. Both rs1800795 and rs1800797 are located in the promoter region of *IL-6* and genetic polymorphisms in this region increase susceptibility of cervical cancer and prognosis.[17]

Environmental factors also influence the risk of cervical cancer. Our study demonstrated that women who used intrauterine devices and barrier methods (cervical cap, diaphragm, female condom) of contraception had a significantly higher risk of developing cervical cancer ($p = 0.013$). This finding is similar to a previous study of ours with a different cohort of cervical cancer patients.[40] Previous studies reported a high prevalence of cancer among workers in the synthetic rubber industry suggesting that the soft silicone and synthetic latex used in these products may have some carcinogenic properties, contributing to cervical cancer.[44] Interestingly, a meta-analysis reported a lower chance of cervical cancer with IUD use.[45] Another study found that women who used Copper IUD had a lower risk of

high-grade cervical neoplasms than levonorgestrel-releasing intrauterine system (LNG-IUS) users.[46] Further investigations are needed to clarify the exact mechanism of how intrauterine devices and barriers contribute as risk factors for cervical cancer.

Our analysis of the SNPs with different clinicopathological characteristics revealed interesting results. We found that smokers were more carriers of the risk allele C of rs1800795 ($p = 0.044$) and risk allele A of rs1800797 ($p = 0.039$). This is similar to a previous study by Zidi et al., who reported a significant association between CC genotype of rs1800795 and smoking in Tunisian cervical cancer patients.[18] Moreover, a recent meta-analysis with Japanese women also reported a significant association between smoking and cervical cancer.[47] The exact mechanism of smoking in increasing the risk of cervical cancer is not known. However, a recent meta-analysis suggested that smoking increases the risk of cervical abnormalities.[48] A previous study with healthy volunteers found that carriers of C allele at rs1800795 may suffer particularly from cigarette smoking and smokers with C allele had higher leukocyte and lymphocyte counts.[49] This polymorphism also influences endothelial function and detrimental effect of smoking is more evident in persons with CC genotype.[50] It is well known that smoking is an HPV cofactor for the development of cervical cancer.[51] But in our cohort, all the patients were non-HPV-infected cervical cancer patients. So, future studies need to be carried out to find out how smoking influences cervical carcinogenesis.

Additionally, we found that the risk allele A of rs1800797 was more prevalent in patients with first-degree relatives with cancer. So, women with A allele at rs1800797 and with a family history of cancer are more prone to develop cervical cancer. Future studies with larger sample size and different ethnic background need to be performed to ensure this result.

There were limitations to our study. HPV-infected cervical cancer patients were not included in our study. So, we cannot confirm if similar findings will be observed in patients infected with HPV. Moreover, our relatively small sample size may be not enough to elucidate robust genotype-disease interaction. We had no gene or protein expression data, so we could not find if polymorphism of the two SNPs has any cis-regulatory effect on gene or protein expression.

Despite limitations, our preliminary findings warrant for future cervical cancer-related studies with larger sample size and different ethnic backgrounds.

5 | CONCLUSION

In conclusion, polymorphisms of rs1800795 and rs1800797 of the IL-6 increase the risk of cervical cancer susceptibility in Bangladeshi women. Future studies with larger sample size and different ethnic background are warranted to validate our findings.

AUTHOR CONTRIBUTIONS

Monishita Shaswati: Data curation; formal analysis; investigation; writing—original draft. **Fihima Hossain Oeishy**: Data curation; formal analysis; investigation; writing—original draft. **Sadia Biswas Mumu**: Formal analysis; investigation; resources. **Md Zahidul Islam Zahid**: Formal analysis; investigation; resources. **Murad Hossain**: Investigation; resources; software. **Md Aminul Haque**: Resources; writing—review and editing. **Hasan Mahmud Reza**: Supervision; writing—review and editing. **Md Shaki Mostaid**: Conceptualization; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

TRANSPARENCY STATEMENT

The lead author Md Shaki Mostaid affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its Supporting Information. All authors have read and approved the final version of the manuscript. Monishita Shaswati and Fihima Hossain Oeishy had full access to all of the data in this study and took complete responsibility for the integrity of the data and the accuracy of the data analysis.

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SUPPORTING INFORMATION

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