Association of Methylenetetrahydrofolate Reductase C677T with Cervical Cancer in Eastern Uttar Pradesh

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Abstract

Globally, cervical cancer(CC) is the second most common cancer among women, and persistent infection with high-risk human papillomavirus (HPV), is associated with the causes of cervical cancer along with host genetic factors. Methylenetetrahydrofolate reductase is a major enzyme in folate metabolism and is required for several biological processes. The MTHFR gene's mutations may cause the enzyme's activity to decline. The functional single-nucleotide polymorphism (SNP) C677T in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene can reduce enzyme activity and alter the chemosensitivity of tumour cells. The present study aimed to look into the role of MTHFR gene polymorphism in the aetiology of cervical cancer in Eastern Indian women. In a case-control study, biochemical analysis was carried out using a commercially available ELISA kit.

In contrast, genetic analysis (PCR-RFLP of peripheral leucocytes) was carried out on all women with cervical cancer and controls. Statistical analysis was done by using the student "t-test" for quantitative variables and the chi-square test for nominal variables, and the odds ratio & 95% confidence interval was calculated the odds ratio and 95% confidence interval to estimate risk. The frequencies of MTHFR C677C, C677T, and T677T were 27.62, 57.62, and 12% in the cervical cancer subjects and 95, 5, and 0.00% in the controls, respectively. Folate and homocysteine levels showed statistically significant differences between these two groups; however, an increasing trend of homocysteine levels was associated with an increasing rate of cervical cancer. We found a negative correlation between homocysteine and the biochemical parameter in the cases. In conclusion, our data suggest that the MTHFR C677T polymorphism, low folate, and vitamins B12 and B6 are associated with cervical cancer and act as "risk factors".

1. INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide. According to the 2022 statistical analysis report, the prevalence is 14,100 new cervical cancer cases and 4,280 deaths among women (1–2). Various factors, such as the number of pregnancies, oral contraceptives, and smoking (3-5), are associated with the development of cervical cancer. In contrast, a few main factors like "Human papillomavirus (HPV)" and "Host-Genetic Structure" are mainly involved with a high risk of cancer (6). Genetic variables and various genes are being researched and may have a role in cervical carcinogenesis (7-8).

MTHFR, the gene that encodes the methylenetetrahydrofolate reductase enzyme, is involved in the development of many clinical manifestations (9) and catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (10-11). Several studies have found that adequate folate levels act as a co-enzyme required for D.N.A. synthesis or methylation and that an abnormal D.N.A. methylation process is linked to cancer (8). Deoxythymidine monophosphate (dTMP) is a precursor for the substrate "5, 10-methylenetetrahydrofolate" and is involved in D.N.A. synthesis and repair. Otherwise, an inadequate level of dTMP may be linked with mutation (8–9). However, low folate intake causes hyperhomocysteinemia (Hhcy), and Hhcy is involved in the pathogenesis of a multitude of clinical conditions, including cervical cancer (4), neural tube defects, mental retardation, and heart disease (5-8). However, it also regulates the expression of oncogenes and the synthesis of "purine and thymidylate," both of which are necessary for D.N.A. repair (8). Although numerous studies have positively supported the linkage between H.C.T. and low folate in cervical cancer and other types of cancer, the association between cervical cancer and the MTHFR C677T polymorphism has been investigated in various population groups. Since there is no evidence of eastern Indian women, we designed the study to investigate a possible correlation between cervical cancer, its biochemical parameters, and the MTHFR C677T polymorphism in eastern Indian women.

2. MATERIALS AND METHODS

Subject

This present case-control study was designed to include n = 69 women aged 25–48 years with cervical cancer from both the O.P.D. of the Department of Obstetrics & Gynecology of Hind Medical College, Ataria and S.S. Hospital, I.M.S., B.H.U., Varanasi. Controls (n = 92) were randomly selected from patients who requested general health examinations in the same hospital during the same period and had no history of cancer. A few biochemical and genetic analyses have been done in the Faculty of Ayurveda at I.M.S., B.H.U., and Varanasi, and some biochemical analyses were carried out at Hind Medical College. The institutional medical ethics committee approved the present study, and all patients and controls enrolled after signing their written informed consent. All Indian female patients newly diagnosed with primary cervical cancer in the hospital between April 2019 and December 2019 were invited to face-to-face interviews within two months after diagnosis. All cases recruited in this study were histologically confirmed. For the same study, overnight fasting blood samples were

collected from all subjects and placed into two anticoagulant tubes. One tube was stored at four °C for genomic D.N.A. isolation. Another difference was that the tubes were immediately placed on crushed ice, shielded from light, and centrifuged at 2000 x g for 10-15 minutes to separate the plasma and then stored at -80 °C until homocysteine, folate, and vitamins B12 and B6 were measured. The study was dually approved by the ethical committee of the Institute of Medical Sciences, B.H.U., Varanasi, and samples were collected after written consent either from the patients or their attendants.

Biochemical tests
A commercially available ELISA kit (Cloud Clone Crop for folic acid, AbnovaTM for vitamin B12 and homocysteine, and Uscn Life Science Inc. in Wuhan for vitamin B6) was used to measure plasma levels of folic acid, vitamin B12 and B6, and homocysteine.

MTHFR C677T polymorphism analysis
Genomic D.N.A. was isolated from peripheral blood, as mentioned previously by Miller et al. (17), and genetic materials were kept at -20 oC till further analysis. The details of forward and reverse primers for MTHFR C677T and its genomic sequences were documented here (F5'TGAAGGAGAAGGTGTCTGCGGGA3') and (R5'AGGACGGTGCGGTGAGAGTG3'). We have developed PCR-specific strategies in a total volume of 25 l containing 50–100 ng of D.N.A., 20 mol of each primer, 200 M of each dNTP, and mixing them with Taq buffer (10 mM Tris HCl pH 8.3, 50 mM KCl), 3.0 mM MgCl2, and three units of Taq polymerase (New England Biolabs). Restriction fragment length polymorphism (RFLP) analysis was carried out to determine the missense mutation in the presence of HinfI (15). PCR product (6 l), digested at 37 oC for three hrs. in a reaction volume of 25 l containing 1U of Hinf-I restriction enzyme (New England Biolabs) and NEB buffer (2.5 l). The RFLP digested product was separated on 3% agarose gel, stained with EtBr, and visualised using the Gel Doc system.

Statistical analysis
All the values were calculated as mean S.D. (standard deviation). The person's correlation calculated the correlation of total homocysteine with other biological parameters. The mean values obtained for the different subgroups were compared using one-way ANOVA. The Hardy-Weinberg equilibrium equation determined genotype and allele frequencies in study cases and controls. Statistical analysis was done using the "X2 test" and compared with controls and the depressive group of patients. The odd ratio at the 95% confidence interval was calculated to determine the genetic risk factors between patients and their respective control study subjects.

3. RESULT

The case–control association study was carried out with 69 cervical cancer patients and 92 controls. Table 1 shows that the average ages of cases and controls were 29.02 5.57 years and 29.62 5.87 years, respectively, with no significant differences in age or body mass index (B.M.I.) observed between cervical cancer patients and healthy individuals. The anthropometric variables and biochemical variables (folic acid (ng/ml), homocysteine (mol/L), vitamin B12 (pg/ml), and vitamin B6 (nmol/L) of the present study (cervical cancer

patients) were compared in Table 1, showing the statistically significant differences between cases and controls.

Table 2 shows the Pearson correlation of homocysteine versus folic acid, vitamin B12, and vitamin B6 and finds a trend toward significant negative correlations between plasma "homocysteine levels" and folate levels, vitamin B12 levels, and vitamin B6 levels determined in the study. The coefficient between "homocysteine and folic acid" was -0.058 in subjects compared to 0.49 in controls, indicating that homocysteine levels increase with the decrease in folic acid in subjects. An inverse correlation was observed between vitamin B12 and B6 and homocysteine levels in both cases and controls.

This study showed evidence of variation from the "Hardy-Weinberg equilibrium" among the patients and control groups. Genotypic and allelic frequencies of "MTHFR C677T" in cervical cancer and control are shown in Table 3, and a higher level of heterozygosity, i.e., 57.62%, was observed in patients than in controls; a statistically significant difference was observed concerning MTHFR genotypic frequencies. The frequencies of the 677C variant allele in cervical cancer and controls were 0.54 and 0.96, respectively. Also, the T677T genotype distribution showed a significant difference between the control and case groups (Table 3). Considering the 677CT+677CC genotype combination as a reference, an extremely significant association was observed for any of the combinations examined (Table 3). The sample size used in the study had sufficient statistical power (>90%) to detect a possible association. The CT genotype, on the other hand, was 24.83 times more common in cervical cancer than in controls.No homozygous mutant (T.T.) genotype was found in the controls, but 12% of the women had cervical cancer.

In some cases, the anthropometric and biochemical parameters were compared about the genotypes (CC, CT, and T.T.) (Table 4). In women with cervical cancer, the level of plasma folic acid was higher in the wild-type (CC) genotype (7.570.28 ng/ml) compared to both genotypes (C.T. and T.T.). Still, there was no significant difference between CC and C.T., whereas it was extremely significant with T.T. (CC/TT). Similarly, homocysteine was higher in the mutant homozygous (T.T.) genotype (11.13 1.06 mol/L) as compared to the normal (CC) genotype (10.72 1.76). The differences between wild homozygous (CC) and heterozygous were present but not significant, while significant differences occur only between CC and T.T.

Table 1: Demographic, anthropometric and biochemical parameters of the cases and controls

Variable	Cervical cancer (N=69)	Control(n=92)	Pvalue
Age(years)	29.02 ± 5.57	29.62 ± 5.87	NS
BMI(kg/m2)	26.89±0.42	26.59±1.87	NS
Folicacid(ng/ml)	7.13±0.67	8.55±0.77	<0.0001*
Homocysteine(μmol/L)	11.13±1.77	10.53±0.87	0.02*
VitaminB12(pg/ml)	327.35±39.84	485.14±67.95	<0.0001*

VitaminB6(nmol/L)	36.57±5.61	48.29±1.24	<0.0001*

Table 2: Correlationbetweentotalhomocysteineandbiologicalparameters.

Biologicalparameters	Case(n=569)		Control(n=91)	
	rvalue	Pvalue	rvalue	Pvalue
Folate(mcg/L)	-.058	581	.049	0.65
VitaminB12(pg/ml)	-.137	0.297	-.253*	0.014
VitaminB6(pg/ml)	-.161	.224	-.110	0.28

Table 3: Genotypedistribution and allelefrequency ofMTHFRC677T
polymorphismincervicalcancerandControls)alongwith the Oddsratioand95%CI.

Genotype	Cervical cancerN=69	ControlN=92	Chi-square	OR95%CI	p-value
CC	32(27.62)	86(95)	74.58	0.02(0.006-0.069)	<0.001
CT	30(57.62)	5(5)	50.53	23.72(7.64-76.17)	<0.001
TT	7(12)	0(0)	14.77	Inf(2.93-inf)	<0.001
C	66 (0.54)	(179)0.96	80.28	0.03(0.01-0.09)	<0.001
T	52(0.47)	(5)0.02	50.54	18.39(6.68-57.80)	<0.001
CT+CCversT.T.	59(39%)	9(61%)	12.86	34.426(1.9-60.4)	<0.005
TT+CTversCC	59(41%)	86(59%)	85.63	45.69(26.70-77.03)	<0.001
CTversCC	50(35%)	91(65%)	59.87	36.55(12.4-17.7)	<0.001

Table 4: Anthropometric and biochemical parameters of cervical cancer according to
thegenotypesCC,CTandT.T. ofMTHFRgeneC677TpolymorphismParameters.

Parameter	CC(N=32)	C.T. (N=30)	T.T. (N=7)
Age(years)	25.13±1.99	26.25±4.53	29.65±5.18
B.M.I. (kg/m2)	26.72±0.34	26.84±0.47	27.45±0.28
Folicacid(ng/ml)	7.42±0.56	7.33±0.27	6.65±0.85
Homocysteine(μmol/L)	10.52±1.85	11.05±1.97	11.13±1.06
VitaminB12(pg/ml)	331.24±45.53	323.64±43.41	307.24±34.14
VitaminB6(pg/ml)	40.78±5.8	39.51±5.50	35.6±4.87

4. DISCUSSION

Epidemiological studies suggest this gynaecological cancer (cervical cancer), one of the most frequent malignancies, is a major public health issue in developing and developed countries. 8,9. In this present study, we have undertaken a case-control study to investigate the role of the anthropometric and biochemical parameters along with MTHFR (C677T) gene polymorphisms and their susceptibility to cervical cancer. In the present study, age and body mass index (B.M.I.) were normal for the cervical cancerous subject compared to a healthy female and were not risk factors for cervical cancer. Numerous studies found the deficiency of food supplements or folate, along with micronutrients such as vitamins B12 and B6, associated with the development of several clinical conditions and cancer. 1,10,11. A large number of studies support the critical role of folate in human health. In contrast, low folate is linked to an increased risk of neural tube defect, other clinical conditions, and several cancers such as colon, breast, cervical, and so on (1, 5, 911, 13). Compared to the control, our current studies' results show the same association finding between biochemical measures of low folate and low Vitamin B12/B6 status and risk of cervical cancer.

Folate deficiency induces hyperhomocysteinemia, which induces genetic instability because low folate decreases D.N.A. methylation, which is a nearly universal feature of early tumour genesis. Although low D.N.A. methylation may promote carcinogenesis by suppressing proto-oncogenes, it is associated with a higher misincorporation of uracil into D.N.A. and an increased incidence of micronuclei in peripheral lymphocytes, and it is also positively correlated with homocysteine levels in the blood. However, cervical cancer has been developed through the activation of a large number of different oncogenes, including MTHFR, an enzyme that plays a crucial role in the folate metabolic pathway due to its involvement in both D.N.A. synthesis and D.N.A. methylation. Interestingly, we have observed genetic susceptibility in various tumours in the last year, leading to an increased focus on MTHFR C677T gene polymorphisms and associated risk in tumorgenesis10.

As we know, the association between MTHFR and cancer susceptibility arises through folate metabolism (D.N.A. methylation processes dependent on S-adenosyl-methionine (S.A.M.)) and thymidylate synthesis, which contribute to D.N.A. replication and cell division. A large body of evidence supports the reduced activity of MTHFR, which decreases the methylation of homocysteine to methionine and, in turn, the level of S.A.M., resulting in D.N.A. hypomethylation. Low levels of the MTHFR substrate, 5,10-methylene-THF, required for thymidylate synthesis, on the other hand, could result in uracil misincorporation into D.N.A., decreased D.N.A. repair, and an increase in the frequency of chromosomal breaks and damage, which are derived from the malignant cell and involved in rapid cell division.12 MTHFR has two common low-function polymorphic variants: the T variant at nucleotide 677 (677 C T) and the C variant at nucleotide 1298 (1298 A-C). MTHFR 677 CT genotype exchanges highly conserved alanine for valine (677CT, alanine valine), resulting in reduced activity of this enzyme, whereas MTHFR 677 TT genotype leads to elevated homocysteine levels and D.N.A. hypomethylation in folate-depleted subjects. However, an unbalanced diet makes different variants possible. Although the regulation of homocysteine metabolism is complex and dependent on multiple vitamins (which act as cofactors), MTHFR mutations also affect folate metabolism, resulting in increased homocysteine. (10-14)

Our findings suggest a new link between the MTHFR C677T polymorphism and cervical cancer. The findings of this study assume importance. The discovery of an increased risk of MTHFR C677T polymorphism in cervical cancer and that heterozygous genotype (C.T.) is associated with decreased enzymatic activity and T.T. with the increased enzymatic activity of MTHFR(15-17). Hyperhomocysteinemia suggests that this may cause cervical cancer in women (18). Concurrent measurement of homocysteine and folate levels would have provided more information about the relationship between dietary MTHFR deficiency, MTHFR C677T polymorphism, hyperhomocysteinemia, and cervical cancer. Our findings are supported by earlier studies (19-20).

5. CONCLUSION

In conclusion, the present case-control study observed statistically significant levels of folic acid among cases and controls and also observed that functional polymorphisms of MTHFR-C677T, a key folate metabolism enzyme, were associated with cervical cancer. A mutation in the MTHFR affects the level of homocysteine and D.N.A. damage. A trend was noticed in the association between increasing plasma total homocysteine levels and cervical cancer, although this was statistically significant. Cofactors are required for the one-carbon metabolism pathway to be completed, and vitamins B12 and B6 act as cofactors for the MTHFR enzyme. Theobservation of the present study needs to be confirmed with a larger sample size. As an adjunct to the preliminary findings reported here, we propose to increase the sample size and study the prevalence of the 677 CT MTHFR gene.

Authors' roles
R.D.:-Concept and design; Acquisition of data;Analysis and interpretation of data;Drafting the article; final approval of the version to be published V.R.-.: Concept and design, performance with other authors;R.K., S.S., J.S.:- Data analysis and interpretation of data; revising the article for important intellectual content; final approval of the version to be published

Conflict of interests
The authors declare that they have no conflict interests.

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