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Homocysteine levels are associated with cervical cancer independent of methylene tetrahydrofolate reductase gene (*MTHFR*) polymorphisms in Indian population

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Abstract

Human papillomavirus is considered to be a major aetiological factor but is not sufficient for the development of cervical cancer. Other host factors, including altered homocysteine levels, a functional marker of folate inadequacy, might contribute to the carcinogenic process. Herein we investigated the potential association of homocysteine levels and *MTHFR* polymorphisms with cervical cancer in 203 histologically confirmed cases including 39 precancer cases and 231 healthy controls with normal cervical cytology. Both patients and controls were screened for human papillomavirus infection. We found that homocysteine and consequently cysteine levels were significantly higher in cases, both cancer and precancer ($p < 0.001$) than controls. However, polymorphisms in the *MTHFR* gene (677C/T and 1298A/C) that are reported to modulate homocysteine levels were not associated with disease. Thus, our study establishes an association of total homocysteine levels with the risk of developing carcinoma of the uterine cervix.

Keywords: *Cervical cancer; human papilloma virus; homocysteine; methylenetetrahydrofolate reductase; polymorphism*

Introduction

Cancer of the uterine cervix is the second most common cancer among women worldwide and it is the most common cancer in Indian women (Das et al. 2000). Epidemiological and molecular biological data established an aetiological link between high-risk human papillomavirus (HR-HPV) infection and cervical cancer. In 70–90% of HPV-infected individuals the virus is naturally cleared. However, in a small percentage of patients, persistent infection with HR-HPV such as HPV type 16 and 18 lead to the development of a cervical intraepithelial neoplastic lesion (CIN), a precursor of cervical cancer (Hausen 2002). Therefore, infection with HPV alone is not sufficient for the development of cervical cancer and multiple host factors might be involved in the progression of the disease (Kohaar et al. 2007). Several nutritional factors may affect the persistence of HPV infection influencing the progression of early precancerous lesions to invasive cancer (García-Closas et al. 2005).

Among the nutritional factors, folate deficiency has been postulated to play a key role in carcinogenesis including epithelial cancers such as colorectal and cervical

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cancer (Powers 2005). Various clinicoepidemiological studies have shown that low folate and/or vitamin B_{12} resulting in elevated levels of homocysteine (a marker for disruption of one-carbon metabolism) is associated with cervical cancer and HPV persistence, although a few have yielded conflicting results (García-Closas et al. 2005, Goodman et al. 2000, Alberg et al. 2000). Folate is one of the factors that regulate the conversion of homocysteine to methionine. Deficiency of folate leads to elevated levels of homocysteine and hence could be associated with increased risk of cervical cancer as a functional marker for low folate (Alberg et al. 2000). Homocysteine, a sulphur-containing amino acid, is a key branch point intermediate in the ubiquitous folate–methionine pathway and could potentially be affected by a large and non-specific range of metabolic alterations. Its elevated levels in plasma act as an important risk factor for a number of common diseases (Botto & Yang 2000, Kumar et al. 2005). It has also been associated with increased risk of cancers including colorectal cancer, squamous cell carcinoma of the head and neck, breast cancer and ovarian cancer (Powers 2005, Weinstein et al. 2001, Eleftheriadou et al. 2006, Chou et al. 2007, Corona et al. 1997). Studies indicate that in cases of cervical cancer, it may enhance the effects of other risk factors including HPV infection (Weinstein et al. 2001, Butterworth 2003), thereby playing a role in carcinogenesis. However, some studies found no association with respect to homocysteine levels in cancer (Ozkan et al. 2007, Schernhammer et al. 2007). Increased levels of homocysteine can be attributed to a correspondingly decreased/deficient activity of the enzymes or cofactors involved in the metabolism of homocysteine.

As a majority of the Indian population adheres to a vegetarian diet, it can be presumed that the levels of homocysteine are in general high due to a deficiency in vitamin B_{12}, a micronutrient sourced only from animal products, that is a cofactor for the enzyme methionine synthase which catalyses the remethylation of homocysteine to methionine. The remethylation of homocysteine to methionine is an important step in the metabolic network that regulates the biosynthesis of nucleosides, the methylation of DNA, protein and lipids, and the levels of homocysteine and methionine. One of the enzymes that play a key role in the remethylation process is methylene tetrahydrofolate reductase (MTHFR), which catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyl THF, the methyl donor for methionine synthesis from homocysteine. The potential influence of MTHFR activity on DNA methylation and its synthesis and repair implicates *MTHFR* as a candidate cancer-predisposing gene. Two common single nucleotide polymorphisms (SNPs) within the coding region of *MTHFR* – 677C/T (Val/Ala) and 1298A/C (Gln/Ala) – have been reported to decrease the activity of the enzyme (Weisberg et al. 1998). These two polymorphisms have been extensively studied all over the world and the frequency of these SNPs has been reported to be considerably different in India than the other populations studied (Kumar et al. 2005). Also, the population frequency of both the polymorphisms exhibits heterogeneity in different populations (Kumar et al. 2005). *MTHFR* SNPs, particularly 677C/T, have been found to be implicated in various diseases (Kumar et al. 2005, Unfried et al. 2002), including cancer (Ma et al. 1997). Both the protective and risk associations have been established for the 677C/T polymorphism in different cancers (Ma et al. 1997, Gershoni-Baruch et al. 2000, Shen et al. 2001), while in a few cancers, no association was reported (Shen et al. 2001, Weinstein et al. 2002). However, the effect of *MTHFR* polymorphisms on cervical cancer (Sull et al. 2004, Kang et al. 2005, Gerhard et al. 2003, Rao et al. 2006) and precancer (Zoodsma et al. 2005, Piyathilake et al. 2000, 2007, Henao et al. 2005, Goodman et al. 2001, Lambropoulos et al. 2003) susceptibility remains controversial.

As no well-defined study has been carried out to evaluate the levels of homocysteine in cervical cancer patients and its relationship with functional polymorphisms in the *MTHFR* gene from India, the present study was designed to compare total homocysteine levels (tHcy) across cases and controls, according to HPV infection and genotype.

Materials and methods

Subjects

In a hospital-based case–control study, a total of 203 cases of Indo-Aryan ethnicity were included, comprising 164 invasive carcinoma, 39 cervical precancer (CIN 2 and 3) and 231 controls. The patients were recruited from Lok Nayak Jai Prakash and Safdarjung Hospitals, New Delhi, with histopathologically confirmed precancer/invasive carcinoma of uterine cervix. The mean age of the patients was 49.4 ± 12.4 years and that of the controls was 48.2 ± 10.2 years.

The age- and ethnicity-matched control group consisting of healthy women with no self- or family history of any neoplastic disease and with normal cervical cytology were recruited from the outpatients of the Department of Gynaecology, Safdarjung Hospital, New Delhi, who came for routine check-up. Inclusion criteria included cytologically detected and histopathologically confirmed cervical cancer/precancer cases and cytopathologically normal individuals for controls. Exclusion criteria comprised pregnant/nursing women, any previously treated squamous intraepithelial lesion (SIL)/cancer and malignancy of any other organ. Written

consent was obtained from all the participants and the study was carried out in accordance with the principles of Helsinki Declaration and was approved by the ethics committee of the Institute.

DNA extraction and HPV detection

Genomic DNA was extracted from fresh cervical tissue biopsy samples (patients) and cervical scrapes (controls) by a standard method using proteinase K followed by phenol/chloroform/isopropanol treatment (Sambrook et al. 1989) for HPV screening and polymorphism studies. In addition blood samples were collected from the corresponding cases and controls in EDTA-coated vacutainers and plasma was separated within an hour of collection. The plasma samples were then stored at -70°C for further analysis.

HPV diagnosis was performed by polymerase chain reaction (PCR) amplification using consensus primers MY09 and MY11 (Manos et al. 1989) and further typing was done by PCR using type-specific primers for HPV 16 and HPV 18 (Franceschi et al. 2003).

MTHFR genotype analysis by SNaPShot

We used the SNaPShot approach to genotype the polymorphic loci of *MTHFR* (C677T and A1298C) as employed by Kumar et al. (2005). GeneScan analysis software (ABI, Foster City, CA, USA) was employed to analyse the results.

Analysis of homocysteine, cysteine and folate levels

Plasma levels of homocysteine and cysteine were determined using high-performance liquid chromatography equipped with a fluorescence detector (Agilent 1100) as described earlier (Ji et al. 1995) using a reverse-phase C18 column (5 µM bead size 4.6 × 150 mm from Phenomenex, Torrance, CA, USA). Standard curves were generated with a known amount of homocysteine and cysteine to calculate the concentration of these thiols in the plasma.

A combination of the CPBA (competitive protein-binding assay) and ECL (electrochemiluminescence) assay, implemented on an Elecsys 2010 immunoanalyser (Roche Diagnsotics GmbH, Mannheim, Germany) using the Cobas Elecsys folate kit for determining the folate was used as per the manufacturer's protocol.

Statistical analysis

The data analysis was performed using the computer software Statistical Package for the Social Sciences (SPSS) for Windows (version 12.0; SPSS, Chicago, IL, USA). Genotypes were checked for conformance of Hardy–Weinberg equilibrium and the χ^2 test was employed for analysis of genotype distribution between different study groups. The Kruskal–Wallis and Mann-Whitney *U* tests were performed for quantitative parameters. Haplotypes were constructed from genotypes of two polymorphic markers by using PHASE (http://linkage.rockfeller.edu).

Results

HPV prevalence

In the studied cohort, about 85.2% (173/203) of cases and 0.87% (2/231) of normal healthy controls showed positivity for the HPV DNA sequence. Out of the HPV-positive cases, 98.3% (170/173) were infected with HPV type 16 and the remaining 1.7% (3/173) were found to be positive for HPV type 18. Both HPV-positive healthy controls were found to be infected with HPV type 16. A total of 53.8% (21/39) of precancer cases and 92.7% (152/164) of invasive cases were found to be positive for HPV infection.

Elevated plasma thiol concentration in cervical cancer/precancer cases

Total plasma thiol levels including t-Hcy, total cysteine (t-Cys) were found to be significantly elevated ($p < 0.001$) in cases in comparison with controls (Table 1). These

Table 1. Plasma thiol concentrations (µmol l^{-1}) among cervical cancer cases and controls.

	Precancer ($n=20$)	SD (95% CI of mean)	Cancer ($n=68$)	SD (95% CI of mean)	Total cases($n=88$)	SD (95% CI of mean)	Controls ($n=121$)	SD (95% CI of mean)	*p*-Value[a]
t-Hcy	18.0 (11.7–24.7)	20.8 (12.8–32.3)	16.4 (10.6–24.9)	12.9 (16.4–22.6)	17.3 (11.3–24.9)	15.0 (17.0–23.4)	9.1 (6.5–12.4)	11.5 (9.7–13.8)	<0.0001[b], <0.0001[c] <0.0001[d], 0.61[e]
t-Cys	244.5 (204.2–296.2)	67.4 (217.4–280.4)	205.6 (143.8–307.5)	128.8 (205.9–268.2)	221.9 (150.2–304.0)	117.4 (214.8–264.6)	166.1 (116.5–205.3)	61.0 (154.4–176.4)	<0.0001[b], 0.0001[c] <0.0001[d], 0.19[e]

t-Hcy, total homocysteine; t-Cys, total cysteine.
Median levels (interquartile range) are shown. [a]Mann–Whitney *U* test, [b]precancer vs controls, [c]cancer vs controls, [d]total cases vs controls, [e]precancer vs cancer.

levels were also found to be significantly associated with precancer ($p < 0.001$) and cancer subjects ($p < 0.001$) in comparison with controls. However, no association was established between precancer and cancer.

Association of HPV infection with elevated plasma thiol levels

Evaluation of the data according to HPV status revealed a significantly higher concentration of plasma thiol levels in both the HPV-positive ($p < 0.01$) and HPV-negative cases ($p < 0.01$) than in the controls. Homocysteine level increased from controls to HPV-negative cases and then to HPV-positive cases, although the association was statistically insignificant ($p = 0.17$). No marked difference was obtained between HPV-positive versus HPV-negative cases with respect to total plasma thiol levels.

Folate deficiency has been reported to be a cause for elevated levels of homocysteine but we did not have enough samples left for the measurement of folate levels in these samples. However, we measured the levels of folate in a limited number of cases and controls that were distinct from the samples that we had originally included in the study. We found that the median levels of folate in cancer patients (6.1 ng ml^{-1}) were slightly lower than in the controls (7.2 ng ml^{-1}) although the difference was not statistically significant (data not shown). This may be due to the small number of samples. We are perfectly aware that the levels of folate that we are currently reporting cannot be compared with the homocysteine values that were obtained in the study population as the samples are different. But, considering the limitation, we measured the levels of folate in a separate set of individuals to get an indication of the folate levels in cancer cases and controls.

Association of MTHFR polymorphisms with cervical cancer risk

Genotypes for *MTHFR* 677C/T and 1298A/C loci were found to be in conformance with the Hardy–Weinberg equilibrium in both the cases and controls (Table 2). In cases of the *MTHFR* 677 locus, the percentages of individuals homozygous for the C allele, homozygous for the T allele, heterozygous for the two alleles and with carrier genotype (CT/TT) were found to be comparable among cases and controls. However, the frequency of genotype distribution at the 1298A/C locus was slightly higher in cases than controls although the difference was not significant. Thus, both *MTHFR* 677C/T and 1298A/C loci were not found to be associated with the cases when compared with controls.

In addition, evaluation of data according to disease severity showed no relationship of the genotypes between precancer versus control, cancer versus control and precancer versus cancer (Table 2). Although the frequency of the polymorphic genotype (677CT/TT; 1298AC/CC) was found to be less in precancer cases than that of controls for both 677C/T and 1298A/C loci, the difference did not attain the limit of statistical significance. No polymorphic homozygous individuals (677TT) were found in the precancer cases, while in the cancer cases, the 1298 locus was found to be insignificantly more polymorphic than in the controls but the 677 locus revealed almost similar distribution of genotypes. The data were also analysed separately for the HPV-positive and HPV-negative cases with respect to controls. The results for both groups tended to be statistically insignificant revealing no association of the *MTHFR* polymorphisms with the viral infection (data not shown).

Table 2. Distribution of *MTHFR* 677C/T and 1298A/C polymorphisms among cervical precancerous, cancerous cases and controls.

Genotype	Precancer	Cancer	Total cases	Controls	*p*-Value[a]
677C/T					
CC	28 (71.79)	113 (68.90)	141 (69.46)	161 (69.70)	0.94[b]
CT	11 (28.21)	47 (28.66)	58 (28.57)	65 (28.14)	0.95[c]
TT	0	4 (2.44)	4 (1.97)	5 (2.16)	0.96[d]
CT+TT	11 (28.21)	51 (31.1)	62 (30.54)	70 (30.30)	0.87[e]
Allele C	0.86	0.83	0.84	0.84	
T	0.14	0.17	0.16	0.16	
1298A/C					
AA	15 (38.46)	58 (35.37)	73 (35.96)	85 (36.80)	0.98[b]
AC	20 (51.28)	83 (50.61)	103 (50.74)	119 (51.52)	0.85[c]
CC	4 (10.26)	23 (14.02)	27 (13.30)	27 (11.69)	0.94[d]
AC+CC	24 (61.54)	106 (64.63)	130 (64.04)	146 (63.20)	0.86[e]
Allele A	0.64	0.61	0.61	0.63	
C	0.36	0.39	0.39	0.37	

Number of individuals (percentage) is shown. [a]χ^2 test, [b]precancer vs controls, [c]cancer vs controls, [d]total cases vs controls, [e]precancer vs cancer.

Association between MTHFR polymorphisms and plasma thiol levels

Analysis of independent and interactive effects of these polymorphisms on total plasma thiol levels (t-Hcy, t-Cys) revealed statistically insignificant association between genotypes and thiol levels (data not shown). Interestingly, when the analysis was done within a group it was observed that the polymorphism at *MTHFR* C677T was associated with homocysteine levels in patients and not in controls under the assumption of a recessive model. However, no such association was observed in the case of *MTHFR* A1298C (data not shown).

Minor allele frequency distribution

The frequency of the 677T allele was found to be exactly the same in the cases as in the controls (0.16) while in precancer and cancer cases it was found to be 0.14 and 0.17, respectively. Similarly, the frequency of the 1298C allele in the patients was 0.39 (precancer 0.36 and cancer 0.39) while it was 0.37 in the controls. Minor allele frequency thus revealed no marked difference in cases with respect to control subjects.

Linkage disequilibrium

Combined analysis of both the patient and control groups revealed that alleles for these two polymorphic loci were not in linkage disequilibrium ($D' = 0.78$, $r^2 = 0.07$) with each other. Haplotype construction using PHASE revealed that CA was the most frequent haplotype (0.46) followed by CC (0.37), TA (0.16) and TC (0.01).

No specific haplotype was found to be significantly associated either with cases (cervical cancer/precancer) or with the controls.

Discussion

The public health initiative in the prevention of cervical cancer in the developed countries has become the most successful among all malignant neoplastic diseases because of the availability of cervical cytology screening programmes. But in developing countries including India, it is still one of the major public health problems as screening is either not available or too expensive and it constitutes 16% of the world's annual incidence (Cohen 2005). In spite of the fact that several other risk factors are involved, including early age of marriage, promiscuity, smoking and use of contraceptives, persistent HR-HPV infection has been considered to be the principal aetiological factor. The role of nutritional factors in biochemical interactions that are part of an oncogenic process might facilitate our understanding of molecular mechanism(s) and the natural history of cervical cancer. Nutritional factors may affect the persistence of HPV infection and thereby influence progression of early precancerous lesions to invasive cancer. To our knowledge, this is the first study in Indian cervical cancer patients investigating the possible association between homocysteine and HPV infection together with functional polymorphisms in the *MTHFR* gene, a key member of the homocysteine metabolic pathway. The main objective of the present study was to evaluate the correlation of plasma total homocysteine and cysteine levels with *MTHFR* polymorphisms according to disease severity and HPV-infection status.

In order to account for the circulating folate status, homocysteine, which is a functional marker of low folate and B_{12}, was considered for its role in cervical cancer/precancer risk. Cysteine levels were determined as intracellular homocysteine is converted to cysteine via the trans-sulphuration pathway which in excess is transported to the circulation through various amino acid transporters. Hyperhomocysteinemia has been associated with various infections and diseases including cancer. Our study indicated that homocysteine levels are significantly associated with cases ($p < 0.001$) in both the precancer ($p < 0.001$) and cancer subjects ($p < 0.001$). This is the only study to take into account the association of homocysteine with both precancer and cancer simultaneously. These results are in consensus with prior studies (Weinstein et al. 2001, Ziegler et al. 2002) on invasive cervical cancer and precancer (Thomson et al. 2000). However, there was no correlation between the trend of disease severity, i.e. from precancer to invasive cancer, with the levels.

Evaluation of the data according to HPV status revealed significantly higher concentration of plasma thiol levels including t-Hcy, t-Cys and t-Hcy+t-Cys in both HPV-positive ($p < 0.01$) and HPV-negative cases ($p < 0.01$) compared with controls implying that total plasma thiol levels are associated with cervical cancer independent of HPV infection. However, plasma homocysteine levels, independent of cysteine levels, showed a statistically insignificant increase with viral infection which is in concordance with a study by Thomson et al. (2000) in cervical precancerous lesions where homocysteine enhances the risk of HPV infection (Thomson et al. 2000). However, analysis of independent and interactive effects of these polymorphisms on total plasma thiol levels (t-Hcy, t-Cys and t-Hcy+t-Cys) revealed no association between genotypes and thiol levels.

Serum homocysteine is a sensitive indicator of folate deficiency and aberration in one-carbon metabolic pathways. The established literature has not yet revealed the exact process underlying the association between elevated levels of homocysteine and carcinogenesis. During tumour progression, the rapid proliferation of

tumour cells causes folate depletion and inactivates the methionine synthase-catalysed remethylation reaction, thereby causing methionine dependency to meet the demand of increased protein synthesis and transmethylation reactions in malignant cells (Cellarier et al. 2003). As a result, homocysteine would not be converted to methionine leading to increased levels in the circulation. Hypomethylation of DNA is a key feature of carcinogenesis. This might lead to chromosome instability and reactivation of transposable elements and inappropriate gene activation. Hypomethylation of certain genes in cervical cancer may cause selective growth advantage and facilitate the transformation process (Fowler et al. 1998). In addition, elevated levels of homocysteine were found in rapidly proliferating tumour cell lines and the levels decreased as the cells started dying (Sun et al. 2002). Also, during carcinogenesis, immune system-derived oxidative stress (generation of reactive oxygen species) causing disruption of redox homeostasis of cell and hence vascular damage might also contribute to hyperhomocysteinemia (Lau et al. 2008). However, a few studies have implicated antifolate drugs such as methotrexate in causing a transient increase in plasma t-Hcy in cancer patients because it indirectly blocks methionine synthase by depleting reduced folate. But rapid proliferation of tumour cells, regardless of the folate status under antifolate drug treatment was found to be major reason for elevated circulating t-Hcy in cancer (Allegra et al. 1986) thereby establishing increased homocysteine as a proliferation marker.

Unlike elevated levels of homocysteine, the effect of elevated levels of cysteine has not been studied in detail. However, some recent reports indicate that elevated levels of cysteine might also be associated with complex diseases. Recently it has been shown that coronary artery disease patients in India have elevated levels of cysteine (Kumar et al. 2009). There are also other studies that have reported that, like homocysteine, cysteine might also be associated with cardiovascular diseases (El Khairy 2001). We recently showed that both cysteine and homocysteine are toxic to yeast and inhibit the growth of yeast probably by inducing endoplasmic reticulum stress (Kumar et al. 2006). A study conducted on hyperlipidaemic patients has also shown an association of elevated levels of cysteine with cardiovascular risk (Jacob et al. 1999). However, unlike our results, an inverse association was established for breast cancer risk (Zhang et al. 2003) and cervical dysplasia (Goodman et al. 2000).

Neither *MTHFR* 677C/T nor 1298A/C loci were found to be associated with the cases including both cervical precancer and cancer in comparison with controls, which agrees with the study by Lambropoulos et al. (2003) for the 677C/T polymorphism in Greek women with cervical precancer and invasive cervical cancer. However, the frequency of the *MTHFR* 677T allele was higher in the precancer group than in the control group without any statistical significance. The effect of *MTHFR* polymorphisms on cervical cancer and precancer susceptibility remains controversial. Several studies relating *MTHFR* polymorphisms specifically with cervical precancer revealed both the protective effect (Zoodsma et al. 2005, Piyathilake et al. 2007, Henao et al. 2005) and disease predisposition effect (Goodman et al. 2001, Piyathilake et al. 2000) with respect to the 677C/T polymorphism.

Similarly, among different studies carried out on the effect of the *MTHFR* polymorphism C677T on cervical cancer, one study in Korean women implicated it with cancer risk (Sull et al. 2004), while in the other studies no association was established (Kang et al. 2005, Gerhard et al. 2003, Rao et al. 2006) which is consistent with our results. Only two studies took into account the 1298A/C polymorphism along with the 677C/T polymorphism but revealed no association with risk of cervical cancer (Kang et al. 2005, Gerhard et al. 2003, Rao et al. 2006) further strengthening our findings.

The present study also revealed that both *MTHFR* 677C/T and 1298A/C polymorphisms are not associated with HPV infection This is in agreement with the work of Goodman et al., in which no association was established with respect to the *MTHFR* 677C/T polymorphism and HPV positivity in spite of the fact that the T allele was associated with cervical precancer in Japanese women (Goodman et al. 2001). Gerhard et al. (2003) also failed to show an association between *MTHFR* C677T and infection with HR-HPV while another study by Piyathilake et al. 2000 showed a non-significant increased risk of HPV infection for the 677C/T polymorphism (Piyathilake et al. 2000). But so far no study has been done on the 1298A/C polymorphism and HPV infection.

Our results also showed that the *MTHFR* 677T allele is comparatively rare (0.16) in healthy North Indians, which is comparable to other studies on the North Indian population (Shekari et al. 2008, Kumar et al. 2005). Also the frequency of the *MTHFR* 1298C allele (0.37) is similar to that reported by Wang et al. (2006) on a South Indian population (0.41) and by Angeline et al. (2004) among Tamilians.

Therefore, although there may be an interaction between this common polymorphism of the folate metabolizing gene and risk of cervical cancer, there seems to be no consensus in this regard. The main reason for these conflicting results seems to be the strong gene–environment interaction between folate status and *MTHFR* polymorphism (Lambropoulos et al. 2003). Also, the effect of this polymorphism on cancer risk may be tissue specific in that individuals carrying the 677TT genotype appear to be protected against colorectal cancer, but the situation with respect to cervical cancer is much less clear at present. Since the frequency of the *MTHFR* C677T polymorphism is very low in the Indian

population, our study may not be sufficiently powered to detect a difference in the genotype between cases and controls. Thus, future studies with larger sample size may provide further insight with respect to MTHFR polymorphisms and cervical cancer.

In conclusion, our study establishes an association of t-Hcy levels with the risk of developing carcinoma of the uterine cervix, including both the precancerous and cancerous lesions. Homocysteine, independent of cysteine, is also indicative of HPV infection. Therefore, we feel that the levels of t-Hcy should also be determined to monitor cancer patients, complementing the currently used tumour markers. The present study also proved no association between *MTHFR* polymorphisms with homocysteine levels in our population. We also found no evidence for gene–virus interaction as *MTHFR* polymorphisms were found to be independent of HPV infection.

However, future studies should include similarly designed studies in different ethnic groups with a larger sample size, other polymorphisms in the folate metabolic pathway and address alterations in folate/homocysteine status and various epigenetic changes linked with it.

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