

# Low Serum Folate Level and Increased Risk of Invasive Cervical Cancer in Bangladeshi Women

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## Abstract:

**Objective(s):** The aim of this study was to evaluate the risk of cervical cancer in relation to serum concentrations of folate.

**Materials and methods:** This case-control study was conducted in the Gynaecologic Oncology Division of Bangabandhu Sheikh Mujib Medical University from November 2015-October 2016. For this study 50 patients with invasive cervical cancer were taken as study population and 50 healthy women of VIA or paps negative were taken as control to observe and compare serum folate level. Exclusion criteria were women with conditions associated with low folate level. Outcome variable was serum folate level and a p value of < 0.05 was considered as significant.

**Results:** Most of the participants belonged to age group 35 to 55 years in both the groups. Most of the study cases had low income status than the control group. Maximum patients with cervical cancer took OCP in comparison to control. Majority of the patients with cervical cancer had significantly low level of serum folate (<2.7ng/ml) than those in the control group (2.7-16.1ng/ml), p=000.

**Conclusion:** A significantly low level of serum folate, was observed in patients with invasive cervical cancer. So dietary interventions with folate supplementation might have some role in prevention of cervical carcinoma.

**Key words:** Cervical cancer, Folate.

## Introduction:

Cervical cancer is a disease of significant worldwide morbidity and mortality. There were about 530 000 new cases of and 275 000 deaths due to cervical cancer worldwide in 2008<sup>1</sup>. The burden of cervical cancer is disproportionately high in developing world<sup>2</sup>. In Bangladesh cervical cancer accounted for approximately 7.8% of all gynaecological patients and 70% of all gynaecological malignancies admitted in BSMMU in the year 2007<sup>3</sup>. Though cervical cancer is preventable, yet it is an important cause of disability and death of women in Bangladesh as many women present at late stage of the disease.

It is evident that human papilloma virus (HPV) is a necessary cause of cervical cancer<sup>4</sup>. However, only a small fraction of women infected with HPV will eventually develop cervical cancer. Alteration of host genes that control viral gene expression, integration of viral DNA into the host genome and disruption of cell cycle regulation are required for persistence of the virus<sup>5</sup>. Several cofactors that might facilitate this progression are hormonal factors such as oral contraceptive pill, multiparity, immunologic factors, smoking, and poor nutrition<sup>6</sup>. Epidemiologic studies suggest that dietary factors may influence risk for cervical cancer. Part of the effect of diet may be attributable to the suppressive action of certain micronutrients on HPV infection, particularly carotenoids and folate<sup>6</sup>.

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Interactions appear to exist between folate status, mutations in the folate-dependent enzyme methylenetetrahydrofolate reductase (MTHFR), plasma homocysteine, and HPV-related cervical dysplasia<sup>7</sup>. Lack of folate causes accumulation of homocysteine in the blood. Folate is needed to metabolize homocysteine in one-carbon metabolism. In one-carbon metabolism, homocysteine accepts a methyl group from 5-methyltetrahydrofolate to form methionine in a vitamin B<sub>12</sub> dependent reaction<sup>8</sup>. Elevated levels of homocysteine have been associated with low levels of folate. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the formation of 5-methyltetrahydrofolate, the source of the methyl group needed for homocysteine to be converted to methionine<sup>8</sup>. Thus homocysteine can be degraded in our body which is needed very certain amount and too much of it is dangerous. It has been shown in a study that daily supplementation with folic acid of 0.8 mg/day typically lowers homocysteine level by about 25%<sup>9,10</sup>. This study was carried out to see the level of serum folate in patients with invasive cervical cancer, so that the development and progression of cervical cancer can be prevented by taking the dietary interventions.

#### Materials and Methods:

This case-control study was conducted in the Gynaecologic Oncology Division of the Department of Obstetrics & Gynaecology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from November 2015-October 2016. Study population was patient attending the Gynaecologic Oncology Division of BSMMU with cervical cancer.

For this study 50 patients with invasive cervical cancer were taken as study population and 50 healthy women of VIA or paps negative were taken as control to observe and compare serum folate level. Sample was taken as convenience. The age range of study population was 30-65 years and disease was confirmed by histopathology. The control group was consisted by the healthy population of age range 30-65 years who came for cervical cancer screening and who found VIA or Pap's smear negative. Patients with conditions associated with low level of folate like pregnancy, previously treated cervical cancer and pre-cancer, malignancy of any other organ, diabetes, osteoporosis, cardiovascular, neurological and renal disease, medication with anti-convulsants, BMI  $\geq$  30 kg/m<sup>2</sup>, smoking, alcoholism, drug addiction were excluded from this study. Primary selection of the cases were made from history and physical examination and histopathological report of cervical growth. These primarily selected patients were approached for inclusion in the study as the cases. Detailed information relevant to the study was recorded

in a properly designed pretested data collection sheet. General examination, per abdominal examination and pelvic examination was done for staging of cervical cancer. In selected cases examination under anaesthesia (EUA) was performed to confirm the staging. Social class of participants were assessed by monthly income and educational level of them.

Serum folate was estimated from blood of both control and study population. With all aseptic precaution 4-5 ml of fasting blood (6-8 hour fast) was collected from a convenient vein of a participant with a disposable plastic syringe and was delivered into a clean dry plastic tube. It was kept in standing position till clot formation. Then serum was separated from cells by centrifugation (5 minutes at 3000 rpm) and was stored at -27°C until the estimation of serum folate.

The folic acid level was determined using the ARCHITECT Folate assay, using protocols commonly referred to as Chemiflex. The data was coded as low (<2.7), normal (2.7-16.1) and high(>16.1)

The protocol of the study was approved by the Institutional Review Board (IRB) of BSMMU Dhaka. Informed written consent was taken from all participants prior to study initiation.

Data was analyzed using SPSS version 17 (SPSS Incorporation, Chicago, IL, USA). Outcome variable was serum folate level. The results have expressed as the mean  $\pm$  SD for the continuous variables and the categorical variables as frequency and percentage.  $\chi^2$  test has been used for analysis of all categorical variables. Unpaired  $t$  test was performed for quantitative parameters. P value  $\leq$  0.05 have considered as significant.

#### Results:

Patients' characteristics regarding age, BMI, parity were similar in both cervical cancer group and healthy control group. Most of the participants belong to 35-55 years range group in both cases and control group. About three-fourth of the participants were multiparous in both cases and control group. There was a significant difference in social class. In cervical cancer group low social class were 74% in comparison to healthy women where low social class was 40%. P is <0.05. There was also significant difference in OCP use. Among cervical cancer group pill users were 86% and in healthy women it was only 16% p<0.05. (Table I).

There was a significant difference in serum folate level between two groups of women, which was  $1.52 \pm 2.28$  vs  $7.10 \pm 4.81$  in cervical cancer group and healthy control group respectively. Eighty eight percent (88%) of cervical cancer group had very low level of folate (<2.7ng/ml) but among healthy women only 12% had the same p is <0.05 (Table II).

**Table-I**  
*Patients' characteristics*

Parameter	Case		Control		Significance
	Mean $\pm$ SD		Mean $\pm$ SD		
Age	48.76 $\pm$ 9.50		40.60 $\pm$ 6.48		0.590
BMI	22.06 $\pm$ 2.75		21.02 $\pm$ 2.27		0.575
Parity	Median	(Range)	Median	(Range)	0.110
	N	(%)	N	(%)	
4	4	(2-7)	3	1-6)	
<i>Social class</i>					
High	2	4	24	48	0.001
Middle	11	22	6	12	
Low	37	74	20	40	
OCP user	43	86.0	8	16	0.000

**Table-II**  
*Serum folate level of the study population*

Serume Folate	Study Group		Control Group		Significane
	Mean $\pm$ SD		Mean $\pm$ SD		
(ng/ml)	1.52 $\pm$ 2.28		7.10 $\pm$ 4.81		0.000
	N	%	N	%	
<2.7 ng/ml (Low)	44	88	6	12	
2.7-16.1 ng/ml (Normal)	06	12	43	86	
>16.1 ng/ml (High)	00	00	1	02	

### Discussion:

Cervical cancer is the most common cancer of the female genital tract in our country. The incidence is high in rural areas, where prevention and screening programs are not easily available<sup>11,12</sup>. Epidemiological studies suggest that micronutrients in diet play a significant role in maintaining health and preventing disease, including cancer. The relationship between pathogenesis of cervical cancer and folate was first described by Whitehead et al who reported that administering folic acid reversed megaloblastic changes in cervical epithelium<sup>13</sup>. Butterworth et al extended the finding to cervical dysplasia in 1982<sup>14</sup>. Folate has protective role in the etiology of cervical cancer<sup>15</sup>. Folate's possible protection against cervical cancer is based on its roles in DNA synthesis and repairing damaged DNA<sup>16</sup>. Folate is involved in DNA methylation through which it may influence gene stability and expression<sup>16</sup>.

Several case-control studies have been conducted to investigate the relation between folate and cervical dysplasia or cervical cancer. In this study, fifty (50) patients with histopathologically confirmed invasive

cervical cancer as cases and fifty women having healthy cervix revealed by screening test (VIA or pap) as controls were included. Both cases and control were almost identical in terms of age, parity, occupation, education and BMI, which are the potential confounders that may affect the risk for cervical cancer as well as serum folate level. But significant difference was observed in OCP use and monthly income of the study subjects.

It is reported that cervical cancer has two peaks, one at about 35 years and another at about 50-55 years<sup>17</sup>. In this study most of the participants belonged to age group 35 to 55 years that reflects one of the bimodal incidence of cervical cancer.

Monthly income of cervical cancer patients in this study was also significantly lower than that of control. Most of the cases (60%) had from low monthly income whereas majority of the controls had average to high monthly income. Carcinoma cervix is more prevalent amongst women living in poor condition with a low income status<sup>17</sup>. The difference in monthly income in this study also indicate that cervical cancer patients with low income conditions are associated with

deficient nutrition whereas controls with relatively higher monthly income have improved nutritional reserve with higher level of folate which is protective against cancer.

Oral contraceptive pill use is a risk factor for cervical cancer. There is also high correlation between serum folate and OCP use<sup>18</sup>. The possible confounding effect of OCP use on the assessment of the effect of folate in study groups may happen as 86% of women in cervical cancer patients and 16% of patients in control used OCP and the difference was statistically significant. So, larger sample size is required to eliminate this selection bias.

Several studies have showed that BMI has influence on folate level. Serum folate decreases with increase in the body size<sup>19</sup>. In the present study majority of the participants had BMI between 18.5-25 kg/m<sup>2</sup> and there was no statistical difference between the mean BMI between the two groups.

This study shows that majority of the patients with cervical cancer (88%) had low level of folate (< 2.7ng/ml); whereas in the control group majority of the participants (86%) had normal level of folate (2.7-16.1ng/ml) and it was statistically significant.

These finding correlate with some studies, which found that higher folate level is associated with significantly reduced risk of cervical cancer<sup>20-26</sup>.

So, nutrient supplementation especially folate is necessary in preventing development of cervical pre-cancers and cancers<sup>26</sup>.

#### Limitations of the study:

In this study, women were not tested for HPV infection, which is the necessary cause of cervical cancer. Although some authors of different studies argued that test for HPV infection should be done for precise interpretation of findings, it is revealed that studies taking HPV infection into account do not differ substantially from those studies that did not control for it. Moreover, further studies with large sample size is needed to establish the association between low level of serum folate and invasive cervical cancer.

#### Conclusion:

A significantly low level of serum folate was observed in patients with invasive cervical cancer compared to that in women with healthy cervix. Malnutrition is a risk factor of cervical cancer. Women at risk of developing cervical cancer can be given advice to

increase intake of vegetables. Public awareness regarding intake of fresh vegetables, fruits to increase serum folate level can be created and women of all reproductive age group can be encouraged to take plenty of vegetables, fruits and folic acid supplementation to prevent cervical cancer.

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