Serum concentration of folate, vitamin B_{12} and homocysteine in patients of cervical cancer

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Article Info

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Received: 20 October 2016
Accepted: 6 November 2016
Available Online: 5 December 2016

ISSN: 2224-7750 (Online)
2074-2908 (Print)

DOI: 10.3329/bsmmuj.v9i4.30104

Cite this article:
Ferdous J, Khatun S, Biawas SK, Akter L, Keya KA. Serum concentration of folate, vitamin B_{12} and homocysteine in patients of cervical cancer. Bangabandhu Sheikh Mujib Med Univ J. 1016; 9: 188-192.

Available at:
www.banglajol.info

A Journal of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Abstract

The aim of this study was to evaluate the risk of cervical cancer in relation to serum concentration of folate, vitamin B_{12} and homocysteine. Fifty patients with invasive cervical cancer and 50 VIA (visual inspection of the cervix after acetic acid application) negative cases (control) with age range of 30-65 years were included. Majority of the patients had significantly low level of serum folate and B_{12} than those in the control group. In contrast, serum homocysteine level was significantly high in cases than those in the control group. The Pearson correlation coefficient test showed a significant negative relationship between serum folate, vitamin B_{12} and serum homocyteine. However, there was no significant association between stage of cervical cancer and level of serum homocysteine. In conclusion, a significantly low level of serum folate, vitamin B_{12} and high level of serum homocysteine were observed in patients with invasive cervical cancer.

Introduction

Cervical cancer is a disease of significant worldwide morbidity and mortality. There were about 530,000 new cases and 275,000 deaths due to cervical cancer worldwide.[1] The burden of cervical cancer is disproportionately high in developing world.[2] In the South East Asian region, about half a million people die every year from cervical cancer.[3] Cervical cancer constitutes 70% of all gynecological malignancies in Bangladesh.[4] According to the cancer registry report of National Institute of Cancer Research and Hospital in 2005-07, cervical cancer is the second (21.5%) leading cancer after breast cancer (25.6%) in women in Bangladesh.[5] Cervical cancer accounted for approximately 7.8% of all gynecological patients and 70% of all gynecological malignancies admitted in Bangabandhu Sheikh Mujib Medical University in the year 2007.[6] Though cervical cancer is preventable, yet it is an important cause of disability and death of women in Bangladesh.

The evidence is increasing that human papilloma virus (HPV) is necessary cause of cervical cancer.[7] Several strains of HPV are oncogenic, with HPV-16 the most prevalent. However, only a small fraction of women infected with HPV will eventually develop cervical cancer. Additional steps involving viral DNA persistence, 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.[8] Several cofactors that might facilitate this progression are hormonal factors such as oral contraceptive use, parity, immunologic factors, smoking, infection with other pathogens and poor nutrition.[9] 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.[9]

Interactions appear to exist between folate status, mutations in the folate-dependent enzyme methylenetetrahydrofolate reductase, plasma homocysteine, and HPV-related cervical dysplasia.[10]

Homocysteine is a sulfar containing amino acid that is normally present in all cells of the body. Our body needs a certain amount of homocysteine in order to function properly. But too much of it is very dangerous. Low level of nutrients like folate, vitamin B_{12} may cause homocysteine to accumulate in the blood. These nutrients are 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_{12} dependent reaction.[11] Elevated levels of homocysteine have been associated with low levels of vitamin B_{12}

and folate. Methylenetetrahydrofolate reduc-tase catalyzes the formation of 5-methyl-tetrahydrofolate, the source of the methyl group needed for homocysteine to be converted to methionine. A common polymorphism in the MTHFR gene, reduces enzymatic activity, limits remethylation of homocysteine and thus raises circulating homocysteine levels.[11]

Thus, elevated homocysteine could be a sensitive biomarker of inefficient one-carbon metabolism caused by insufficient folate, vitamin B_{12}, or by genetic variation in critical pathway enzymes. Because of difficulties with the current assays for serum and red blood cell folate, serum homocystene may be a preferable biomarker for folate inade-quacy.[12] Ziegler et al suggested that elevated homocysteine could be a better measure of inadequate folate nutritional status than direct measurement of circulating folate and may be related to cervical carcinogenesis.[12] Daily supple-mentation with folic acid of 0.8 mg/day typically lowers homocysteine level by about 25% and the addition of vitamin B_{12} lowers it by a further 7%.[13,14]

This study was carried out to see the association between serum folate, serum vitamin B_{12}, and serum homocysteine in patients with cervical cancer, so that the development and progression of cervical cancer can be prevented by taking the interventions that could reduce the level of homocysteine.

Materials and Methods

This study was conducted from July 2015 to June 2016. Study population was the patients of cervical cancer attending the Gynecologic Oncology Division of the Bangabandhu Sheikh Mujib Medical University. Fifty cases and 50 control were included in the study by purposive sampling. Regarding the inclusion criteria, cases were histopathologically confirmed patients with cervical cancer between the ages of 30-65 years and control was women with normal cervix (VIA negative) with age range of 30-65 years. Exclusion criteria was women with conditions associated with elevated homocysteine like pregnancy, previously treated cervical cancer and pre-cancer, malignancy of any other organ, women with diabetes, osteoporosis, cardiovascular, neurological and renal disease, women with medication with anticonvulsants, women with BMI >30 kg/m^2, smoker, alcoholic and drug addict. Outcome variables were serum folate level, serum vitamin B_{12} level and serum homocysteine level. Regarding the study procedure, the protocol of the study was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University. Primary selection of the cases was made from the history and physical examination and finally confirmed by histopathological report of the cervical growth. These primarily selected patients were approached for inclusion as the cases. Women who came to VIA center of Bangabandhu Sheikh Mujib Medical University for cervical cancer screening and diagnosed VIA negative were selected as controls. Informed written consent was taken from all participants prior to study initiation. Detailed information relevant to the study was recorded in a properly designed pretested data collection sheet. General examination and pelvic examination were done for the staging of cervical cancer. In selected cases, the examination under anesthesia was performed to confirm the staging.

Blood sample collection, processing and preser-vation

With all aseptic precaution, 4-5 mL of fasting blood (6-8 hours fast) was collected from a convenient vein of the study subject with a disposable plastic syringe and was delivered into a clean dry plastic tube. It was then kept in standing position till the clot formation. Then serum was separated from the cells by centri-fugation (5 min at 3,000 rpm) and was stored at -27^{o}C until the estimation of serum folate, vitamin B_{12} and homocysteine levels.

Laboratory test

The vitamin B_{12} and homocysteine estimation were done by micropartical enzyme immunoassay (MEIA) method (AxSYM- Abott, USA). Bound homocysteine (oxidized form) was reduced to free homocysteine that was enzymatically converted to S -adenyl homocysteine (SAH) which was measured for quantitative data. Serum folate estimation was done by Ion Capture Assay method (AxSYM- Abott, USA).

Statistical analysis

Data was analyzed using SPSS version 17 (SPSS incorporation, USA). The results have expressed as the mean ± SD for the continuous variables and the categorical variables as frequency and percentage. Pearson chi-square test has been used for analysis of all categorical variables. Unpaired t'-test was per-formed for quantitative parameters. P value <0.05 have considered as significant.

Results

The mean age of the patients with cervical cancer was 48.8 years and that of the control group was 40.6 years. Most of the participants belong to 35-55 years range group in both cases (70%) and control group (72%). Regarding parity, most of the participants had para 3-4 in both cases (74%) and control (84%) group (Table I). Seventy percent of the

Table I

Sample characteristics

	Case (n=50)	Control (n=50)
Age (Year)		
<35	8	10
35-55	35	36
>55	7	4
Parity		
≤2	2	4
3-4	37	42
≥5	11	4
BMI		
<18.5	4	6
18.5-25	38	39
>25	8	5
Education		
Illiterate	15	22
Literate	35	28
Occupation		
Housewife	45	40
Service	4	9
Others	1	1
Monthly income (US$)		
<130	7	4
130-260	30	16
260-400	11	6
>400	2	24

Table II

Serum folate, vitamin B_{12} and homocysteine levels

	Case (n=50)	Control (n=50)
Serum folate		
<2.7 ng/mL	44	6
2.7-16.1 ng/mL	6	43
>16.1 ng/mL	0	1
Serum vitamin B_{12}		
<239 pg/mL	45	4
239-931 pg/mL	5	46
>931 pg/mL	0	0
Serum homocysteine		
<4.5 µmol/L	0	7
4.5-15 µmol/L	10	42
>15 µmol/L	40	1

participants were literate in cases and 56% in control group. Most of the participants were housewives in both cases (90%) and control group (80%). About 60% women had monthly income between US$ 130-260 in cases whereas 48% women had monthly income US$ >400 in control group and it was statistically significant (p = 0.001).

The use of oral contraceptive pill was remarkable in patients (86%) whereas only 16% in case of control group. This was statistically significant (p = 0.000). Majority of the pati -ents had BMI in between 18.5 to 25.

Table II shows that majority of the patients (88%) had low level of folate (<2.7 ng/mL). In case of control group majority of the participants (86%) had normal level of folate (2.7-16.1 ng/mL) and it was statistically significant (p = 0.000). Similarly, in majority of the cases (90%) level of serum B_{12} was low (<239 pg/mL). In contrast it was normal (239-931 pg/mL) in most of the participants of the control group (92%) which was also statistically significant.

Serum homocysteine level was high (>15 µmol/L) in most of the patients (80%); in contrast its level was normal (4.5-15 µmol/L) in the majority of the participants of the control group (84%) and this finding was statistically significant (p = 0.000). The mean serum homocysteine level was 25.3 µmol/L in cases of cervical cancer and that of the mean in control group was 8.8 umol/L.

The Pearson correlation coefficient test showed a significant negative relationship between serum folate and serum homocyteine. The correlation coefficient was -0.360. This result means that there is a linear relationship between serum folate and serum homocyteine. The Pearson correlation coefficient test showed a significant negative relationship between vitamin B_{12} and serum homocysteine. The correlation coefficient is -0.537. There was no significant association between stage of cervical cancer and level of serum homocysteine (p = 0.058). The level of homocysteine was same in both operable and inoperable stages.

Discussion

In this study, both cases and control were almost identical in terms of age, parity, occupation, BMI and education which are the potential confounders that may affect the risk for cervical cancer as well as serum homocysteine level. But significant difference was observed in the use of oral contraceptive pill and monthly income of the study subjects. Most of the participants belonged to age group 35 to 55 years. Although invasive cancer of the cervix is reported at all ages, it has two peaks, one at about 35 years and another at about 50-55 years.[15] In this study, cervical cancer patients (mean age, 48.8 years) were older than control (mean age, 40.6 years). The Third National Health and Nutrition Examination Survey in Americans showed that total homocysteine concentration increased with increasing age but participation bias is unlikely in this study as the age distribution of cases was not significantly different from that of the controls.

High parity is a risk factor for cervical cancer and this phenomenon is reflected in this study where 22% of cervical cancer cases had parity >5 in comparison to controls (8%). In a study by Yilmaz et al regarding the relation of parity with homocysteine revealed that the homocysteine levels were positively associated with the number of deliveries.[16] In the present study, cases were not significantly different from control with respect to parity which eliminate the effect of this confounder on the association of homocysteine level and cervical cancer risk.

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.[15] The difference in monthly income in this study also indicate that cervical cancer patients with low income conditions are associated with deficient nutrition which causes elevated level of homocysteine whereas controls with relatively higher monthly income have improved nutritional reserve with lower level of homocysteine which is protective against cancer.

Oral contraceptive pill use is a risk factor for cervical cancer. There is also high correlation between serum homocysteine and the use of oral contraceptive pill.[17] The possible confounding effect of oral contraceptive pill use on the assessment of the effect of homocysteine in study groups may happen as 86% of women in cervical cancer patients

and 16% of patients in control used oral contraceptive pill 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 homocysteine level. Serum homocysteine increases with increase in the body size. In the present study majority of the participants had BMI in between 18.5-25 and there was no statistical difference between the mean BMI between the two groups.

In this study for analysis of serum homocysteine level, the 5th-95th percentile range of homocysteine, (4.5-15 µmol/L) was considered as normal value which is similar to that reported in The Third National Health and Nutrition Examination Survey.[18] In this study it was found that serum homocysteine level was high (>15 µmol/L) in most of the cases (80%); in contrast its level was normal in the control group (84%). Moreover, in this study the mean homocysteine level in cervical cancer patients was higher (25.3 µmol/L) than that of controls (8.8 µmol/L) and this difference was statistically significant inferring that homocysteine levels are significantly associated with cervical cancer.

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

Similar to serum folate level, in majority of the cases (90%) level of serum B_{12} was low (<239 pg/mL); in contrast it was normal (239-931 pg/mL) in most of the participants of the control group (92%) which was also statistically significant. The inverse relationship between folate, vitamin B_{12} and cervical cancer describes the protective role of folate and vitamin B_{12} in the etiology of cervical cancer.

These findings correlate with some studies which found that the combination of higher folate and vitamin B_{12} are associated with significantly reduced risk of cervical cancer.[19-24] Till date, only four studies have examined the association between serum homocysteine and invasive cervical cancer. Among these, results of 3 studies showed a significant increase in cancer risk for women with elevated plasma homocysteine.[25-27] Result of the present study is in consensus with those studies. The 4th study regarding the association of homocysteine and invasive cervical cancer was a small study nested in the Washington County, MD cohort, which included 26 *in situ* and 13 invasive cervical cancer cases. That study also showed positive association of serum homocysteine with increased risk of cervical cancer but result was not statistically significant.[19]

Homocysteine may be more predictive of cervical cancer risk than low folate. In addition to indicating folate inadequacy, homocysteine can be elevated in response to low vitamin B_{12} (necessary for conversion of homocysteine to methionine.[18,28,29] There is also problems in assessing dietary and blood folate status.[12,30]

In the present study, it was also evaluated the association between the clinical stage of cervical cancer and serum homocysteine level. No statistically significant difference in homocysteine level was observed between early stage and advanced stage of cancer in this study although mean homocysteine level (30.8 mg/L) was higher in advanced stage than that in early stage (23.2 mg/L) but this was not statistically significant. These findings were similar to a study done by Weinstein et al that indicates that systemic effect of disease progression did not affect the homocysteine level.[26]

So, nutrient supplementation especially folate, vitamin B_{12} are necessary to reduce homocysteine level in preventing development and progression of cervical pre-cancers and cancers.

Conclusion

A significantly elevated level of serum homocysteine was observed in patients with invasive cervical cancer compared to that in women without cervical cancer.

Acknowledgement

We would like to thanks Research Grants Committee of BSMMU and University Grants Commission for funding this research. We are grateful to all the doctors and stuffs of the department of Biochemistry for their sincere help in conducting the research.

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