Plasma Folate and Vitamin B12 in Patients with Cervical Squamous Intraepithelial Neoplasia

Anwar Noori Al-Basam, Duja Hasan

ABSTRACT:

BACKGROUND:
Cervical squamous intraepithelial neoplasia is a premalignant condition of cervix. Human papilloma virus (HPV) infection has been associated in the development of premalignant and malignant changes in the cervix. Folate and vitamin B12 can potentially influence carcinogenesis through its effect on DNA synthesis or methylation.

OBJECTIVE:
To determine the influence of plasma folate and vitamin B12 concentrations in cervical squamous intraepithelial lesions.

PATIENTS AND METHODS:
A case-control study conducted in Baghdad Teaching Hospital from the 1st of Dec 2014 to the 31st of Dec 2015 whereas 60 patients participated in the current study and divided in two groups: cases whose pap smears showed cervical squamous intraepithelial neoplasia and controls whose pap smear was normal).

RESULTS:
Mean age of cases was 33.6±4.9 years; prevalent age group was 30-40 years (76.7%). No significant difference was observed between cases and controls regarding age (P=0.5), educational level (P =0.6) and oral contraceptive pills (P=0.4), but a significant association was found between cases and controls regarding parity and smoking (0.01 and 0.003) respectively. Moreover significant association was found between both groups of the study regarding to the folic acid deficiency (P <0.001)and vitamin B12 deficiency (P=0.01). Human Papilloma virus was higher among cases than controls but it is still not significant (p=0.2).

CONCLUSION:
There is significant association between folate and vitamin B12 deficiency, smoking and parity with the cervical squamous intraepithelial neoplasia.

KEYWORDS: folate , vitamin B12, cervical squamous intraepithelial neoplasia

INTRODUCTION:

Cervical intraepithelial neoplasia (CIN) is a premalignant cervical disease that is also called cervical dysplasia or cervical interstitial neoplasia or cervical squamous intraepithelial lesions (CSIL).these were the terms used to describe premalignant squamous cervical cellular changes. The trend is now tending towards the use of Squamous Intra-epithelial Lesions.[1]
Cervical squamous intraepithelial lesions (CSIL): is a potentially premalignant transformation and abnormal growth (dysplasia) of squamous cells on the surface of the cervix.[1]CSIL is not cancer, and is usually curable.[2]
Among risk factors for CSIL, human papillomavirus infection, smoking and sex

Baghdad Teaching Hospital.

steroid hormones, in general hormonal contraceptives are the most studied and important. [3]
Low-risk types are rarely found in cervical neoplasia, but some types, in particular HPV 6 and 11 are associated to benign genital condylomas. High risk HPV DNA is detected in 37% in low-grade lesions(CSIL1 and borderline lesions), 89% in high-grade lesions (CSIL2 and CSIL3), and in 40% of cytological CSIL in papsmears, but with no information of histological CSIL grade. [4]
Folate is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement.
Folate functions as a coenzyme or cosubstrate in single-carbon transfers in the synthesis of nucleic acids (DNA and RNA) and metabolism of amino acids and is involved in essential one-carbon transfer reactions that are important in DNA synthesis and replication, cell division, and

growth and survival, particularly for rapidly dividing cells[5].

Vitamin B12 is a water-soluble vitamin that is naturally present in some foods and is required for proper red blood cell formation, neurological function, and DNA synthesis [6].

Lower levels of folate and vitamin B12 in the diet may result in reduced levels of DNA methylation as well as elevated levels of homocysteine which can result in cancer[7]. Indeed it has been shown that low levels of B vitamins can result in reduced levels of DNA methylation, which, in turn may be related to increasing severity of cervical dysplasia and cancer. [8]Folate levels in cervical tissue have been correlated with DNA methylation level in the tissue. It has also been suggested that low folate levels resulting in hypomethylation of DNA and DNA strand breaks may enhance the integration of HPV DNA into human DNA.

AIM OF THE STUDY:
The purpose of this study was to determine the influence of plasma folate and vitamin B12 concentrations in cervical squamous intraepithelial lesions.

PATIENTS AND METHODS:
Study design & settings:

study agreed to participate within the selected criteria were   A case control study conducted in Baghdad Teaching Hospital from the 1st of December 2014 to the 31st of December 2015.

All patients attending to (Early detection of cervical cancer clinic) and Gynecological outpatient clinic at the time of the considered eligible.

A total of 60 women were included in this study and divided into two groups:

Group A:thirty women with pap smear showed Cervical Squamous Intraepithelial Neoplasia.

Group B :thirty women who attended to the same clinic and whose pap smear was normal considered as control group.

Inclusion criteria involve:
Women between the age of 18—40 years old, Women with no previous history of cervical or other lower genital tract premalignant or malignant disease, Currently not pregnant and

who had been pregnant or lactating within the past year and no current use of anti-folate medications such as methotrexate, Sulfasalazine, or phenytoin.

Exclusion criteria involve:
Women with age more than 40 or less than 18,Women who had chronic medical illness such as hypertension, diabetes, epilepsy and sickle cell anemia.

METHODS:
Age, educational status, cigarette smoking and frequency, hormonal/oral contraceptives, parity were ascertained for both groups.

Exfoliated cervical cells collected with a cervical brush and immediately rinsed in 10 mL of Phosphate Buffered Saline (PBS), which were transported to the Central Health laboratory.

Cervical cells DNA was extracted using the QTAampMiniElute Media kit and HPV genotyping test (linear array; Roche Diagnostics) was done according to the manufacturer's instructions .

5ml of venous blood was collected from the study groups in EDTA containing blood collection tubes, samples were clotted at room temperature, centrifuged at 3000 degree and frozen at -20, were kept cold until transported in ice to the laboratory, The plasma folate was measured using the *L.casei* microbiological assay, plasma vitamin B12 using a competitive radio-binding assay (SimulTRAC-SNB, MP Biomedicals).

Statistical analysis:
Chi-square used for categorical variables and Independent Samples t-test was used to compare between two means. In all statistical analysis, level of significance (p value) set at ≤ 0.05.

We categorized plasma folate levels into sufficient (5.21-20)Ng/ml and insufficient (<5.21)Ng/ml and vit. B12 into sufficient ($\geq$200.6 pg/mL)and into insufficient(<200.6 pg/ml)

RESULT:
A total of 60 women were included in present study, 30 women with cervical squamous intraepithelial neoplasia (CSIL) as cases and 30 women with no CSIL (controls).

Table 1: Socio-demographic characteristics and parity of cases and control ,there is statistically significant assosciation between cases and control regarding parity (p=0.01) and smoking (p=0.003) .

Variable	Group A		Group B		$\div^2$	p
	No.	%	No	%		
Age						
20-29 years	7	23.3	9	30.0	0.3	0.5 (NS)
30-40 years	23	76.7	21	70.0		
Total	30	100.0	30	100.0		
Mean±SD	33.6±4.9		32.6±4.9			
Educational level						
Illiterate	13	43.3	11	36.7	1.7	0.6 (NS)
Primary	6	20.0	6	20.0		
Secondary	5	16.7	9	30.0		
Institute/coll	6	20.0	4	13.3		
Total	30	100.0	30	100.0		
Parity						
1	2	6.7	5	16.7	8.5	0.01 (S)
2	4	10.3	12	40.0		
≥3	24	80.0	13	43.3		
Total	30	100.0	30	100.0		
mean±SD	4.2±1.9		2.2±1.2			
Smoking						
Non-smoker	20	66.7	29	96.7	9.0	0.003 (S)
Smoker	10	33.3	1	3.3		
Total	30	100.0	30	100.0		
Oral contraceptive pills						
Positive	6	20.0	4	13.3	0.4	0.4 (NS)
Negative	24	80.0	26	86.7		
Total	30	100.0	30	100		

S=Significant association, NS= Non-significant association.

Table 2: The relation between vitamin B12,folate and HPVin studied groups (cases and control). there is significant assosciation regarding folic acid (p<0.001) and vitamin B12 (p=0.01) between both groups.there is non significant difference in HPV between both groups , although ahigher percentage of HPV in cases compared to controls .

P	÷²	Group B		Group A		Variable
		%	No.	%	No.	
<0.001 (S)	8.8	Folic acid				
93.3	28	56.7	17			Normal
6.7	2	43.3	13			Deficiency
100.0	30	100.0	30			Total
		9.6±6.9		6.2±4.1		Mean±SD
0.01(S)	6.6	Vitamin B12				
86.7	26	56.7	17			Normal
13.3	4	43.3	13			Deficiency
100.0	30	100.0	30			Total
		498.5±519.7		257.8±152.4		Mean±SD
0.2(NS)	1.2	HPV				
33.3	18	73.3	22			Positive
66.7	12	26.7	8			Negative
100.0	30	100.0	30			Total

S=Significant association, NS= Non-significant association.
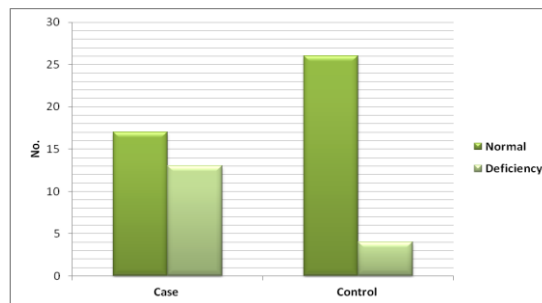

Figure 1: Distribution of vitamin B12 according to the studied groups (cases and control).

Table 3: Distribution of age, parity, folic acid and vitamin B12 means according to the studied group (cases and control).

P	t-test	Group B Mean±SD	groupA Mean±SD	Variable
0.5 NS	0.6	32.6±4.9	33.6±4.9	Age (years)
<0.001S	4.8	2.2±1.2	4.2±1.9	Parity
0.02 S	2.3	9.6±6.9	6.2±4.1	Folic acid (ngm/ml)
0.01 S	0.4	498.5±519.7	257.8±152.4	Vitamin B12 (Pgm/ml)

S=Significant association, NS= Non-significant association.
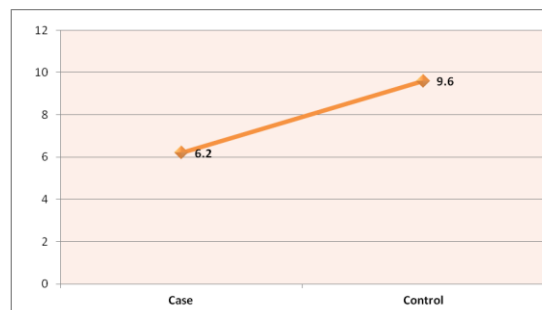
Figure 2: Distribution of folic acid means according to the studied group.

DISCUSSION:

In the current study, the mean age of cases was 33.6±4.9 years, which is less than the mean ages of Barazanjy, et al.[9,] and Alberg, et al,[10.] Studies which is 36.84years and 38years, respectively. Mean parity of CIN patients (cases) in the present study was 4.2±1.9, 80% of them had ≥3 children and with statistically significant association. This is similar to that found by Piyathilake, et al. [11] where the parity was significantly higher in cases than in controls (P = 0.01),]Regarding smoking, this study is concordant with Piyathilake, et al.[12] and Toistrup, et al.[13] studies, there is significant association between smoking and CSIL.Regarding to education and use of oral contraceptive pills, this study is inconsistent with Alberg, et al.[10] study, but is consistent with Binesh, et al., study, there is no association between OCP and CSIL.[14]For the folic acid and B12: the current study found that the mean folic acid level of cases was less than that of the controls and 43.3% of the cases had folic acid deficiency with a statistically significant association found between the studied group. The mean vitamin B12 level of cases was 257.8±152.4 Pg/ml, 43.3% of cases had deficiency in vitamin B12 with a significant difference in vitamin B12 level between CIN cases and controls (p=0.01). This is similar to that revealed by Kwaœniewska et al.,[15] Barchitta, et al. study [16] and Piyathilake, et al.,[12] The current study showed that human papilloma virus (HPV) is higher in case group than that in control group, Still there is no significant association between them (P= 0.2) this is may be due to the small sample size in the present study. This study showed that the decrease of folate and vit. B12 is associated with increase the infection with HPV which is concordant with many previous studies[11,12, 16].

CONCLUSION:

There is significant association between folate deficiency, vitamin B12 deficiency, smoking and parity with the squamous cervical intraepithelial neoplasia.

REFERENCES:

1. Adekunle OO. Cervical Intraepithelial Neoplasia (CIN) (Squamous Dysplasia). Intraepithelial Neoplasia, Supriya?Srivastava/ (ED). 2012:279.
2. American College of Obstetricians and Gynecologists. (ACOG) ObstetGynecol. Cervical cancer in adolescents: screening, evaluation, and management. Committee Opinion. 2010;116: 469–72.
3. Trottier H, Franco EL. Human papillomavirus and cervical cancer: burden of illness and basis for prevention. Am J Manag Care 2006;12:S462-72.
4. Munger K, Baldwin A, Edwards KM, et al. Mechanisms of human papilloma virus induced oncogenesis. J Virol 2004;78: 11451-60.
5. Kelemen LE. The role of folate receptor α in cancer development, progression and treatment: cause, consequence or innocent bystander?. International journal of cancer. 2006 ;119:243-50.
6. National Institutes of health. "Office of Dietary Supplements. nd Dietary Supplement Fact Sheet: Vitamine B12." Dietary Supplements Fact Sheets 2011.
7. Mahmood I Premalignant and Malignant Disease of the Cervix. Edmonds, Keith, ed. Dewhurst's textbook of obstetrics and gynecology. Jhon Wiley & Sons.2011; 8: 747-52.
8. Hernandez BY, McDuffie K, Wilkens LR, Kamemoto L, Goodman MT. Diet and premalignant lesions of the cervix: evidence of a protective role for folate, riboflavin, thiamin, and vitamin B12. Cancer Causes & Control. 2003;14: 859-70.
9. Barzanjy BK, Talat LA, Ismail SA. Cervical dysplasia: assessment and risk factors among women attending the Maternity Teaching Hospital in Erbil, Kurdistan-Iraq.Zanco J Med Sci. 2013;17: 286-293.
10. Alberg AJ, Selhub J, Shah KV, Viscidi RP, Comstock GW, Helzlsouer KJ. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. Cancer Epidemiology Biomarkers & Prevention. 2000 ;9:761-64.
11. Piyathilake CJ, Macaluso M, Alvarez RD, Bell WC, HeimburgerDC, Partridge EE. Lower risk of cervical intraepithelial neoplasia in women with high plasma folate and sufficient vitamin B12 in the post-folic acid fortification era. Cancer Prevention Research. 2009;2:658-64.
12. Piyathilake CJ, Macaluso M, Chambers MM, Badiga S, Siddiqui NR, Bell WC, Edberg JC, Partridge EE, Alvarez RD, Johanning GL. Folate and Vitamin B12 May Play a Critical Role in Lowering the HPV 16 Methylation–Associated Risk of Developing Higher Grades of CIN. Cancer Prevention Research. 2014;7:1128-37.

13. Tolstrup J, Munk C, Lykke Thomsen B, Svare E, van den Brule AJ, Grønbæk M, Meijer C, KrügerKjær S. The role of smoking and alcohol intake in the development of high‑grade squamous intraepithelial lesions among high‑risk HPV‑positive women. Acta obstetrician et gynecologicaScandinavica. 2006;85:1114-19.

14. Binesh F, Akhavan A, Pirdehghan A, Davoodi M. Does oral contraceptive pill increase the risk of abnormal Pap smear?. Iranian journal of reproductive medicine. 2013;11:761.

15. Kwaśniewska A, Tukendorf A, Semczuk M. Folate deficiency and cervical intraepithelial neoplasia. European journal of gynaecological oncology. 1996 ;18:526-30.

16. Barchitta M, Quattrocchi A, Maugeri A, La Rosa N, Scalisi A, Agodi A. Folate deficiency is associated with Cervical Intraepithelial Neoplasia: perspective for prevention. The European Journal of Public Health. 2015; 25:176-82.