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A STUDY OF PLASMA FOLATE LEVELS IN HIV POSITIVE, TREATMENT NAIVE PATIENTS ATTENDING TO ICTC IN TERTIARY CARE HOSPITAL.

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ABSTRACT Comparison of plasma Folate levels in newly diagnosed, treatment naive, HIV positive patients with HIV negative controls, Estimate the plasma Folate levels in patients and controls. Evaluate the Folate levels and its correlation to CD4 cell count. Patients belonging to both sexes attending to outpatient department of general medicine and ART centre in Gandhi hospital, Secunderabad. Controls were healthy volunteers from Blood Bank, Gandhi Hospital. Our aim was to estimate the plasma folate levels in newly diagnosed, treatment naive, HIV positive patients and compare them with their levels in HIV negative controls and also, correlate them with the CD4 cell count. For this, we have studied Hemoglobin and serum folic acid levels in 50 HIV positive patients, who are not on HAART and 50 healthy controls. CD4 cell counts were estimated in the HIV positive patients. In HIV positive patients, 24 (48%) were males and 26 (52%) were females and in controls, 26 (52%) were males and 24 (48%) were females. Mean age of HIV positive patients was 30 years and of controls was 26 years. There was no statistically significant difference in the male female ratio and age distribution at baseline level in the test and control group.

KEYWORDS : HIV, Plasma folate levels

INTRODUCTION

The human immunodeficiency virus (HIV) is a lenti virus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Multiple nutrient deficiencies occur relatively early in the course of HIV infection. They occur due to malabsorption, altered metabolism, gut infection, altered gut barrier function, chronic diarrhoea, anorexia, impaired nutrient storage, etc. The prevalence of specific nutrient abnormalities is widespread among HIV-infected patients compared to non-HIV patients. Several observational studies have reported that a number of micronutrient deficiencies among HIV-infected individuals are associated strongly with a faster progression to disease and death and raise the possibility that normalization might increase the interval of symptom-free survival. HIV-positive patients appear to require an intake of these nutrients in multiples of recommended dietary allowances when compared to normal individuals. [1]

Micronutrients such as Vitamin B12 and folic acid, whose deficiencies are found in higher numbers in HIV-infected patients and their deficiencies may typically present initially with neuropsychiatric disturbances, including depression, dementia, and demyelinating myelopathy.

Folate is an essential vitamin required for vital biochemical reactions in the human body, which includes one-carbon transfer reactions, and the formation of purines and pyrimidines for DNA and RNA synthesis. [2] Lack of folate has been undoubtedly linked to a number of disorders which includes carcinogenesis and anemia among others. [2] These in HIV-infected individuals could be a source of additional morbidity and increased mortality.

The role of folate in carcinogenesis has been well studied in colorectal cancers.[2] Epidemiologic studies have also associated folate deficiency with cancers of the lung, esophagus, stomach, brain, cervix, pancreas, and breast, and with leukemia. [3, 4] It has been proposed that because folate is required for the synthesis of deoxythymidylate from deoxyuridylate. [5] The mis-incorporation of uracil during DNA synthesis could be a possible mechanism for carcinogenesis in folate deficiency. Similarly, it has also been proposed that alterations in DNA methylation due to folate deficiency could contribute to carcinogenesis by modulating gene expression.[6] Folate may affect immune cell proliferation and responsiveness due to its crucial role in nucleotide synthesis. [5]

Proliferation of various cell types has also been reported in folate deficiency. [7–9] Cells lacking folate have been shown to accumulate in the S phase due to nucleotide imbalance and slow DNA synthesis; it has also been reported that such cells also have increased uracil mis-incorporation and DNA damage. [9, 10] Documented studies have shown that when folate is added back to these folate-deficient cells, there is a reversal of the S phase accumulation, and proliferation is restored. [9,10]

In human beings, folate deficiency has been shown to reduce the proportion of circulating T lymphocytes and their proliferation in response to mitogen activation, which in turn decreases resistance to infections. [11] There is also the association of folate deficiency with faster disease progression after infection of T lymphocytes by HIV type 1 virus. [12, 13] Risk of acute lymphocytic leukaemia is said to increase with low folate status. [14,15]

Anemia in HIV-infected individuals occur in 10 to 20% at presentation and are diagnosed in 70 to 80% of patients over the course of the disease, [16, 17] thus making it more common than thrombocytopenia or leukopenia in patients with AIDS. The etiology of anemia in HIV infection remains largely unclear. In recent years, several attempts have been made to elucidate the mechanisms leading to HIV-associated anemia. Direct infection of erythroid progenitors have been discussed, [18] but has not been proven. Furthermore, soluble factors like HIV proteins and cytokines have been suggested to inhibit growth of hematopoietic cells in the bone marrow of HIV-infected patients. So far, no emphatic statement could be made whether these factors are directly involved in myelosuppression or mediate their effect by inhibiting growth factor synthesis. [18] Other implicated factors include changes in cytokine production with subsequent effects on hematopoiesis. [19] Most of the studies on anemia in HIV infection have been focused on iron studies, with less emphasis on folate levels. Folate is rapidly depleted in diseases, especially when there is prolonged anorexia, increased metabolic rate, and high cell turnover. [20] The HIV virus is known to have a rapid turnover and can generate up to ten billion virion in 24 hours [21], and the implication for this is a rapid depletion of folate stores. Plasma folate level ranges from 3 to15 µg/l. [20] The human daily requirement approximates to 50 µg daily in infancy, to about 100 µg daily in adults.[20] The actual amount is determined by metabolic and cell turnover rate. The total body folic acid stores are 5 to 10 ml with a half-life of about 0.7 hours. [2, 20] It is widely and rapidly distributed throughout the body. Large amount is stored in the liver as methylated folic acid (34%), while about 66% is bound to plasma proteins. [2, 22] 20 to 90% of ingested folic acid is excreted as folinic acid in the urine within 24 hours. [2,22]

Hence, we have studied the serum folic acid levels in newly diagnosed HIV positive patients, who are not on ART therapy and its correlation

to anaemia (Hb%) and CD4 cell count.

AIM :
Comparison of plasma Folate levels in newly diagnosed, treatment naive, HIV positive patients with HIV negative controls. .

OBJECTIVES :
Estimate the plasma Folate levels in patients and controls. Evaluate the Folate levels and its correlation to CD4 cell count.

MATERIALS AND METHODS
STUDY DESIGN: Cross sectional study

SAMPLE SIZE AND DURATION OF STUDY:
50 patients and 50 controls
November 2016 to December 2017

SOURCE OF DATA:
Patients belonging to both sexes attending to outpatient, department of general medicine and ART centre in Gandhi hospital, Secunderabad. Controls were healthy volunteers from Blood Bank, Gandhi Hospital.

INVESTIGATIONS REQUIRED:
1) HIV serology,
2) CD4 counts,
3) Serum Folic acid
4) Complete Blood Picture

INCLUSION CRITERIA:
Subjects having HIV serology positive by all the 3 kits of Micro ELISA, Comb AIDS-RS and HIV Tri-dot antigen kit.

Age more than 18 years

EXCLUSION CRITERIA: HIV patients on ART
Who are newly diagnosed in the stage of AIDS
Who are having severe anemia and Known CKD Patients
Who are on Anti Folate drugs
Who are on Multivitamin supplementation

METHODOLOGY:
A minimum of 50 patients attending to department of general medicine OPD and ICTC of Gandhi hospital between November 2016 to December 2017 and satisfied both the inclusion and exclusion criteria, were enrolled in the study.

- All subjects had HIV serology +ve by the 3 tests.
- Informed consent had been obtained from all the subjects and controls.
- CBP, Serum folic acid levels, and CD4 counts were estimated in the subjects and CBP, Serum folic acid levels [by CLIA method] were estimated in the controls.
- Results were analysed and correlation of serum folic acid with CD4 counts was studied.

RESULTS

Comparison of serum folate levels between HIV positive and HIV negative subjects

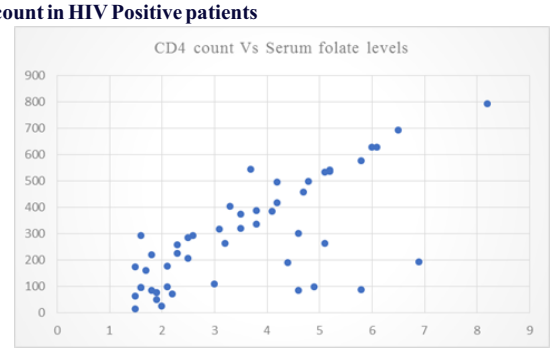
	Number of subjects	Mean (SD) ng/ml	95% CI		F	Sig. (p-value)
			Lower Bound	Upper Bound		
HIV positive	50	3.59 (1.69)	3.11	4.08	80.735	0.000*
HIV negative	50	10.68 (5.01)	8.61	12.75		

*Statistically significant p<0.05

Correlation between Serum folate levels and CD4 count in HIV positive patients

		Serum folate	Cd4 Count
Serum folate	Pearson Correlation	1	721**
	Sig. (2-tailed)		000
	N	50	50
CD4 Count	Pearson Correlation	721**	1
	Sig. (2-tailed)	000	
	N	50	50

Scatterplot showing relation between serum folate levels and CD4 count in HIV Positive patients



(X axis= Serum folate levels in ng/ml, Y axis= Cd4 cell count)

Comparison of Hemoglobin levels between HIV positive and HIV negative subjects

	Number of subjects	Mean (SD) ng/ml	95% CI		F	Sig. (p-value)
			Lower Bound	Upper Bound		
HIV positive	50	9.55 (2.54)	8.82	4.08	20.888	0.000*
HIV negative	50	11.96 (0.95)	11.57	12.75		

*Statistically significant p<0.05

Correlation between Serum folate levels and Hb levels in HIV positive patients

		Hb levels	Serum folate
Hb levels	Pearson Correlation	1	.472**
	Sig. (2-tailed)		.001
	N	50	50
Serum folate	Pearson Correlation	.472**	1
	Sig. (2-tailed)	.001	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

DISCUSSION

Our aim was to estimate the plasma folate levels in newly diagnosed, treatment naive, HIV positive patients and compare them with their levels in HIV negative controls and also, correlate them with the CD4 cell count. For this, we have studied Hemoglobin and serum folic acid levels in 50 HIV positive patients, who are not on HAART and 50 healthy controls. CD4 cell counts were estimated in the HIV positive patients. In HIV positive patients, 24 (48%) were males and 26 (52%) were females and in controls, 26 (52%) were males and 24 (48%) were females. Mean age of HIV positive patients was 30 years and of controls was 26 years. There was no statistically significant difference in the male female ratio and age distribution at baseline level in the test and control group.

Mean serum folate level in the test group was 3.59 ng/ml (SD 1.69) and in the control group, the mean value was 10.68 ng/ml (SD 5.01). The values were statistically significant with p value <0.05.

In HIV positive test group, there was a strong positive correlation (r value = 0.721) between the CD4 cell count and serum folic acid levels. The serum folic acid levels were much low in the HIV positive patients whose CD4 count was <200 cells/µl, when compared to the patients whose CD4 count was >200 cell/µl. Out of the 50 patients in the test group, 19 had a CD4 cell count less than 200/ µl and the rest (31) had a count >200 cells/µl. The average serum folic acid level in patients with a CD4 <200 was 2.79 ng/ml, which is much lower than folic acid level 4.09 ng/ml in the patients with CD4 count >200.

Many studies from around the world have documented persistently high occurrence of anaemia in HIV infected persons, especially as the disease progressed.[16,17] This has been attributed to various factors like poor dietary intake, effect of chronic inflammation, and opportunistic infections.[23,24] However, a large percentage of asymptomatic HIV infected persons have shown early occurrence of anaemia even in the absence of attributable etiological factors.[16] **Friis et al.,**[23] in a study of 1,669 subjects in Zimbabwe, recorded a significantly lower plasma folate levels among the HIV-positive study group. The study showed a

significant association between low plasma folate levels (<3 ng/ ml) and falling values of CD4 cell count of the subjects studied; 69.7% of these subjects with plasma folate level below 3 ng/ml had CD4 cell counts below 200 cells/µl (p = 0.0004;OR, 2.92). Our study is in line with the above study, with serum folic acid levels 2.79 ng/ml in patients with CD4 cell count <200/ µl. These findings are in tandem with reports by Courtemanche et al. [24] who described higher CD4 apoptosis in moderate level of folate deficiency. The study also reported DNA damage and maturation arrest in the S-phase of the cell cycle which was reversible on folate repletion. Folate status is known to modulate immuno-competence and resistance to infections and to affect cell-mediated immunity by reducing circulating T-lymphocytes and decreasing response to mitogen activation.[25] Therefore, a combination of these mechanisms in a folate deficient immuno-compromised host could result in rapid deterioration and faster progression to AIDS.

Mean haemoglobin value in HIV positive patients was 9.55 gm/dl (SD 2.54) which is statistically significantly low (p value= 0.00) when compared to a 11.96 gm/dl (SD 0.95) haemoglobin level in healthy controls. 29 patients (54%) out of 50 had Hb levels below 10 gm/dl. Our results are consistent with **Castro et al.**, [26] who studied HIV-infected asymptomatic subjects in Southern Brazil and reported a prevalence of 41%. The mean Hb 8.38 gm/dl (SD 2.6) in patients with CD4 counts <200cells/µl, is statistically significantly low (p <0.05) when compared to the mean Hb 10.38 gm/dl (SD 2.2) in patients with CD4 count >200cells/µl.

There is a medium positive correlation (r value 0.472) between Hb levels and folic acid levels in the HIV positive patients. patients in the test group with haemoglobin level >12 gm/dl (12) had a mean serum folic acid level 4.13 ng/ml (SD 2.1) and in those with Hb <12 gms (38), the mean serum folic acid level was 3.42 ng/ml (SD 1.5). This relation between the Hb levels and folic acid levels is statistically insignificant (p value >0.05) and can be supported by the fact that anaemia in HIV-positive subjects is multifactorial.

Akanmu Alani et al. [27] conducted a study in 100 patients of HIV positive and 100 healthy volunteers and the mean ages for control and study group were 38 ± 2.3 and 32 ± 1.7 years, respectively. Mean plasma folate concentration among the study group (5.04 ng/ml) was significantly lower than that for the control group (15.89 ng/ml; p = 0.0002). Prevalence of anaemia among the study group was 72% (144 of 200), with a mean haemoglobin (Hb) concentration of 9.5 g/dl compared with mean Hb of 13.0 g/dl among the control group (P = 0.002). Plasma folate levels correlated positively with CD4 cell count (r = 0.304, P<0.05).

Our study results are consistent with this study with mean plasma folate concentration among the study group (3.59 ng/ml) being significantly lower than that in the control group (10.68 ng/ml; P = 0.00). Incidence of anemia (Hb<10 gm/dl) among the study group was 58% (29 of 50) with a mean haemoglobin (Hb) concentration of 9.55 g/dl compared with mean Hb of 11.96 g/dl among the controls (p = 0.00). Plasma folate levels correlated positively with CD4 cell count (r = 0.721).

SUMMARY AND CONCLUSION

- The study showed a significant association between low plasma folate levels and falling values of CD4 cell count of the subjects studied.
- Low folate levels associated with anemia in HIV positive subjects.
- Our study concludes that folate deficiency occurs as an early event in HIV infection, and that the introduction of folate prophylaxis may be justified as soon as HIV diagnosis is confirmed to delay disease progression and early occurrence of anemia.
- Further large studies might needed to demonstrate that routine folic acid supplementation is desirable in patients infected with the human immunodeficiency virus.

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