Original Article

Urine methylmalonic acid levels in HIV-infected adults with peripheral neuropathy

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Abstract

Background: Cobalamin deficiency and peripheral neuropathy (PN) are commonly seen in HIV-infected adults. The level of urine methylmalonic acid (UMMA), a reliable indicator of tissue cobalamin status, was determined in HIV infected subjects with and without PN to establish this association.

Methods: One hundred and ninety-eight (198) consenting HIV infected subjects with and without PN were recruited for the study. UMMA level was determined by Cation Exchange High Performance Liquid Chromatography (HPLC) with Ultraviolet detector in 165 subjects. Simple proportions of patients with raised UMMA (defined as value> 3.4 mg in 24hr) were determined for each arm.

Results: Among the 198 subjects studied, 146 had PN and 52 had no PN. From the 165 subjects whose UMMA was studied, raised UMMA was found in 76.6% (36 of 47) of subjects with no PN as compared with 53.4% (63 of 118) of those with PN (p=0.018).

Conclusion: Cobalamin deficiency (measured by UMMA level) even though common in HIV infected subjects, may not be the cause of peripheral neuropathy in these subjects.

Keywords: Peripheral Neuropathy, Cobalamin deficiency, Urine methylmalonic acid (UMMA), HIV infection

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Peripheral nerve disorders are frequent complications of HIV disease. Distal symmetrical polyneuropathy (DSP) is a common peripheral nerve disorder associated with HIV and it is found in more than one third of these patients (1). This may be secondary to HIV (HIV-DSP) or due to antiretroviral drug toxicity (2). Predisposing factors for DSP include increased age, cobalamin (vitamin B12) deficiency, alcohol exposure, HIV viral load 'set point' and low CD4 cell count (3). The etiopathogenesis of HIV-associated DSP is unknown; implicated factors are neurotoxic effects of cytokines, toxicity of HIV proteins, and mitochondrial damage (1). HIV infection decreases both cobalamin, haptocorrin and holotranscobalamin levels and the clinical abnormalities of gastrointestinal function associated with abnormal Schilling tests provide a mechanism for cobalamin depletion in this patient population (4). HIV-infected patients are at risk of developing cobalamin deficiency due to decreased intake of nutrients as a result of reduced appetite, loss of nutrients from diarrhea or from vomiting due to infection or antiretroviral (ARV) drug toxicity and due to abnormal cobalamin metabolism (5, 6). Estimation of UMMA has been found to be of great value in the diagnosis of cobalamin deficiency (7). Except for a rare life threatening enzyme deficiency (methylmalonyl- coA mutase deficiency) which is evident early in life, cobalamin deficiency is the only known cause of high UMMA. Normal patients excrete only traces of UMMA (0-3.4 mg/day) (8). Norman considered it a "gold standard" test for tissue cobalamin deficiency (9). This is because cobalamin is required for the transfer of methyl group to homocysteine to form methionine a precursor of S-adenosyl methionine (SAM) which is the crucial methyl donor in numerous reactions involving proteins, phospholipids, and biogenic amines.

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Its deficiency causes impairment of methionine synthesis causing pathological changes in the peripheral nerves, optic nerves, and posterior and lateral columns of the spinal cord and the brain resulting in neuropathy, myelopathy, myeloneuropathy, dementia and cognitive impairment, cerebellar ataxia, optic atrophy, and psychosis and mood disturbances. (10). This study was carried out to determine whether deficiency of cobalamin is associated with neuropathy among adult HIV-infected subjects using UMMA level.

Methods

A cross -sectional study was done at the HIV clinic of the Lagos University Teaching Hospital. All consenting HIV infected adults with signs and symptoms of neuropathy and those with no neuropathy were recruited between October 2009 and April 2010. The participants were aided to fill a structured questionnaire including demographic information, history of ARV and other drugs and clinical features of neuropathy. Inclusion criteria was the presence of paraesthesia. Features of paraesthesia assessed included pain and/numbness, sensory loss in the feet or fingers including glove and stocking distribution pattern of sensory impairment, abnormal muscle tone, power or reflexes, loss of coordination, loss of joint position and vibration senses; abnormal gait and Romberg's sign. The subjects with other common causes of peripheral neuropathy such as diabetes, alcoholism, or those on medications like phenylbutazone, chloramphenicol and others were excluded from the study.

Collection of samples: Twenty-four hour urine sample was collected into two litre containers for UMMA quantitation. Urine samples were stored at -20^{o}C until analyzed. UMMA was quantified using cation-exchange High Performance Liquid Chromatography (HPLC), (Agilent) series 1100 (Japan) with ultraviolet detector (wavelength 230nm); ODS hypersil column (reverse phase C18 and length 250 mm by 4.6 mm) with particle size of 5 micron and an ambient temperature (11). The HPLC's mobile phase was acetonitrile/ 8 mmol H_2SO_4 (ratio 20/80%), flow rate was 0.7/ml with a manual injector (20 µl loop) and isocratic elution. Drug history and CD4 cell count results were retrieved from the subjects' case files. To serve as control, twenty apparently healthy HIV negative subjects (10 males and 10 females) were recruited for UMMA assay. The descriptive data were given as means ± standard deviation (SD). The chi-square test was used for analytical assessment. The differences were considered statistically significant when a p value obtained was < 0.05.

Results

One hundred and ninety-eight subjects with the mean age of 39±12 years were evaluated. There were 125 females with mean age 38±11yrs and 73 males with mean age 41±12 yrs. The females were younger than the males (p=0.047). From the 198 subjects, only165 had their UMMA values determined because of non-compliance of some participants to instructions on urine collection. The normal range of UMMA used in this study is 0-3.4 mg/24hr (8). Sixteen of the 20 control subjects had no detectable UMMA. Four had detectable UMMA levels (9.5 mg/24hr; 13.1 mg/24hr; 15.6 mg/24hr; and 18.8 mg/24hr). The overall mean value of this group was 2.84±6.02 mg/24 hr.

As shown in table 1, 165 subjects had their UMMA value determined. From this 165, 118 had PN while 47 had no PN. From those with PN, 53.4% (63 of 118) had raised UMMA (>3.4 mg/24hr), while 46.6% had normal UMMA (<3.5 mg/24 hr).

From the 47 who had no PN, 76.6% (36 of 47) had raised UMMA, while 23.4% had normal UMMA. Furthermore, those with normal UMMA 83.3% (55 of 66) had PN while 16.7% (11 of 66) had no PN. There was a significantly higher proportion of subjects without neuropathy with raised UMMA (p=0.018).

Table 1. Peripheral Neuropathy and Urine MMA

Urine MMA	Neuropathy	No Neuropathy	Total
0-3.4 mg/24hrs	55	11	66
3.5-20mg/24hrs	27	18	45
>20mg/24hrs	36	18	54
Total	118	47	165

The mean UMMA in subjects with neuropathy was 29.5±71.3 mg/24 hr with a median of 6.6. This value was lower than the mean obtained for subjects without neuropathy (49.8±78.9 mg/24 hr) with median of 13.1. However, this difference was not statistically significant (p=0.118). The data on treatment status and UMMA is shown in table 2.

From the 165 subjects who had their UMMA determined, 37 subjects were treatment naïve. From this 37, 81% (30 of 37) had raised UMMA while 7 (19%) had normal UMMA. From the 127 treatment experienced subjects, 68 (53.5%) had raised UMMA while 59 (46.5%) had normal UMMA. More treatment naïve subjects had significantly higher UMMA values (p=0.001).

Table 2. Treatment Status and UMMA

Treatment Status	UMMA (mg in 24hrs)			
	0-3.4	3.5-20	>20	Total
ART naïve	7	9	21	37
ART experienced	59	37	32	128
Total	66	46	53	165

The mean UMMA (mg/24hr) of treatment naïve subjects (57.3±72) was also significantly higher than that of treatment experienced subjects (28.9±73.7) (p=0.026). As enumerated in table 3, from 198 subjects screened for neuropathy, 146 (73.7%) had neuropathy and higher mean CD_4 cell count/mm^3 (327.7±223.6) than 59 subjects without neuropathy with mean CD_4 of (299.6±156). The difference was however not statistically significant. From 146 who had neuropathy, 116 were treatment experienced with mean CD_4 cell count of 352.5±223.1 and 30 were treatment naïve with a mean CD_4 cell count of 248.7±209.6. CD_4 cell count of treatment experienced subjects was significantly higher than the treatment naïve subjects (p= 0.02).

From the 52 subjects without neuropathy, 37 were treatment experienced and they had a mean CD_4 cell count of 295.1±222.8. Fifteen were treatment naïve, had a mean CD_4 cell count of 299.6±156. There was no statistically significant difference between treatment naïve and treatment experienced subjects without neuropathy (p=0.9).

Table 3. Mean CD_4 cell count versus Neuropathy and Treatment Status

Treatment	Neuropathy Present		Neuropathy Absent	
	Naïve	Experienced	Naïve	Experienced
N	30	116	15	37
Mean	248.6	352.48	299.6	295.14
Std Dev	209.55	233.12	155.95	222.76
Median	192	319	270	269
Std Error	38.2584	22.7721	40.2661	36.6215
95% CI	210.3416 to 286.8584	307.8467 to 397.1133	220.6784 to 378.5216	223.3618 to 295.14
P	0.021		0.934	

Discussion

Urine MMA reflects tissue or cellular cobalamin levels and UMMA excretion is a sensitive indicator of cobalamin deficiency. Many reports have confirmed that UMMA estimation is an easy and sensitive first diagnostic test to be used in patients suspected to have cobalamin deficiency (7, 12). If UMMA is above reference value, cobalamin levels can be determined for further diagnosis (17). The overall prevalence of cobalamin deficiency as indicated by raised UMMA is 60% (99 of 165). Though with figures much lower than what was obtained in this study, this high prevalence is in agreement with findings of other researchers on this subject (13-17). Wood et al. reported an incidence of about 20% whether a person was receiving protease inhibitors or not (13). More studies have confirmed that vitamin B_{12} depletion was also found in subjects infected with HIV who were asymptomatic leading to the idea that vitamin B_{12} depletion could serve as an early marker for HIV infection (15, 16). In this study, a higher proportion of subjects without neuropathy had raised UMMA, 36 of 47 (76.6%) than those with neuropathy 63 of 118 (53.4%). The mean UMMA in these subjects (49.8±78.94) was also higher than in subjects with neuropathy (25.51±71.28).

This shows that cobalamin deficiency as indicated by raised UMMA may not be the major cause of neuropathy in HIV patients since raised UMMA is common in all HIV infected patients whether they have neuropathy or not. This agrees with Trimble et al. who had concluded long ago that vitamin B_{12} deficiency is not a cause of HIV associated neuropathy (17). This view was indirectly supported more recently by Sanjay et al. who listed neuropathological

damage by HIV infection itself, antiretroviral drugs and lactic acidosis syndrome as major causes of neuropathy in HIV infection (18). No mention was made of cobalamin deficiency as a major cause. In this study, the patients with neuropathy (n=146) generally had a higher mean CD4 cell count than those without neuropathy (n=52) although this did not reach statistical significance. Furthermore, when those with neuropathy were grouped into ART naïve and experienced, those on treatment who had neuropathy had significantly higher mean CD4 cell count (352.5±223.1) than ART naïve with neuropathy (248.6±209.6). These findings may suggest that neuropathy may be a complication of ART in those subjects with neuropathy and higher CD4 cell count. There is enough evidence from reports that many NRTI particularly stavudine may cause neuropathy (19, 20).

In this study, neuropathy was not influenced by the course of HIV disease as some patients had symptoms that predated hospital attendance and neuropathy was a finding in all stages of the disease.

In conclusion, although cobalamin deficiency measured by raised UMMA may be common in HIV-infected patients, it cannot be validly held responsible for neuropathy in many of these patients. Limitations of the study**:** Diagnosis of peripheral neuropathy was clinical as nerve conduction studies and electromyography (EMG) were not done.

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Conflict of Interest: The authors declare no conflict of interest.

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