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PREVALENCE AND PATTERNS OF THE VARIOUS HEMATOLOGICAL MANIFESTATIONS IN PATIENTS OF MYELONEUROPATHIES

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Abstract

Background: Patients of neuroloical syndrome (Myeloneuropathy, Myelopathy and Neuropathy) show various haematoloical manifestations. Complete clinical and early Laboratory evaluation help in early identification of these cases and help in prevention of permanent neurological damage.

Material and methods: 81 Patients of myeloneuropathies attending the outpatient's department neurology were taken. Detailed hematological, biochemical investigations and bone marrow examination was carried-out in all patients as per the protocol.

Results: 81 cases of neurological syndrome were taken these were classified as myeloneuropathy (32), myelopathy (11) and neuropathy (38). Of these 81 patients, 55 (67.9%) were males and 26 (32%) were females (male: female ratio 2.1:1). Peripheral blood examination, bone marrow examination and biochemical evaluation was done in all. Anemia, present in 48 patients (59.2%), Macrocytosis was defined as MCV ≥100 fl, was present in 38 patients (46.9%). Most common finding on bone marrow examination was reversal of myeloid and erythroid ratio (M:E ratio) and was present in more than three-fourth of patients (76.5%). Megaloblastic changes in erythroid cells were present in 42 patients (51.8%). Biochemical tests revealed increased serum LDH in 56.5% of patients. Out of eighty one patients, twenty eight patients (34.5%) were found to be Vitamin B12 deficient.

Conclusions: This study included 81 patients presenting with various neurological syndromes myelopathy, myeloneuropathy and neuropathy with associated myelocognitive and neuropsychiatric features to study the hematological manifestations such as anemia, other cytopenias, macrocytosis, neutrophil hypersegmentation and megaloblastic changes in the bone marrow.

Introduction

Myeloneuropathies are a group of disorders of varying etiology affecting the spinal cord and neurons (sensory and motor neurons). Non-compressive myeloneuropathies constitute about one third of cases and the etiology includes nutritional myelopathy, acute transverse myelitis, multiple sclerosis, HIV myelopathy etc. Nutritional myelopathies are commonly observed in developing countries since appropriate nutrients essential for optimal functioning of the central and peripheral nervous system may be lacking in the diet. Vitamin B12 deficiency is the most common cause of nutritional myeloneuropathies and affects all age groups [1, 23,].

Vitamin B-12 deficiency may take several years to develop and patients may be asymptomatic or may present with a wide spectrum of hematological and neuropsychiatric manifestations. The main systems affected due to vitamin B12 deficiency are the hematological, skin and mucous membranes, and the nervous system. Neurological manifestations may be the earliest and often the only manifestations of cobalamin deficiency. The neurological syndromes associated with vitamin B12 deficiency include myelopathy, neuropathy, neuropsychiatric abnormalities, and less often extrapyramidal syndrome, seizures and optic nerve atrophy

The spinal cord affliction called subacute combined degeneration (SACD) is clinically characterized by symmetric dysaesthesia, disturbance of the position sense, and spastic paraparesis or tetraparesis. Identification of vitamin B12 related neurological syndromes is important since early identification and treatment may help in reversing the neurological damage and late interventions may be associated with incomplete recovery and sequelae.

The hematological manifestations of cobalamin deficiency include megaloblastic hematopoiesis with characteristic peripheral blood and bone marrow findings. Diagnosis of vitamin B12 deficiency requires demonstration of abnormalities in the peripheral blood and/or bone marrow and low serum Vitamin B12 levels [1,21,30,] Hematologic and neurologic manifestations of vitamin B12 deficiency vary amongst patients. Several issues related to the biochemical

basis of megaloblastic hematopoiesis and associated neurological damage and/or dysfunction remain unresolved . Vitamin B12 deficiency associated neurological syndrome may also occur without development of anemia and other hematological abnormalities [21,] The inverse correlation between severity of hematological manifestations and neurological damage is also reported but the reason is not well understood [21,] Although most patients respond well to cobalamin treatment, the response to the hematologic derangements is prompt and complete, neurologic response is more variable and residual neurological abnormalities may persist in many patients.

Diagnostic advances have led to early recognition of B12 deficiency with subtle hematologic or neuropsychiatric manifestations, however patients diagnosed at a late stage may have significant degree of anemia and other cytopenias with varying degree of neurological impairment. This study was undertaken to evaluate the prevalence and patterns of the various hematological manifestations in patients of myeloneuropathies seen at our hospital and to relate the hematological findings with severity of neurological dysfunction in various neurological syndromes.

Material and Methods:

Patient selection

A total of 81 consecutive cases that presented with clinical manifestations suggestive of myelopathy, myeloneuropathy and neuropathy, both newly diagnosed patients and some patients under follow-up were also included in the study. The study was conducted with informed consent of the patients and the study protocol was approved by the institutional ethical committee.

Clinical study and examination

Complete demographic details, clinical history and neurological examination findings at the time of inclusion in the study were recorded on a clinical record proforma. Patients were subjected to a detailed clinical history, with recording of symptoms with duration, dietary habits, smoking, alcohol intake, previous surgery and major illnesses including diabetes, thyroid disorders, arthritis etc. Additional details included regarding any alterations in social behavior, neuropsychiatric manifestations, sleep disorders, addictions etc. were also noted.

The general examination findings anaemia, jaundice, skin pigmentations or oedema, organomegaly etc were recorded. A detailed neurological examination was carried out in each patient. Motor examination included evaluation of muscle power, tone, tendon reflexes, joint position and vibration were tested. coordination and in sensory system examination, sensations to pinprick, touch, temperature. Complete Laboratory workup was done including peripheral blood examination, bone marrow examination and biochemical evaluation.

Results:

The study included 81 patients that presented with various neurological syndromes, myelopathy, myeloneuropathy and neuropathy with or without neuropsychiatric manifestations. Of these 81 patients, 55 (67.9%) were males and 26 (32%) were females (male: female ratio 2.1:1). Age of the patients ranged from 14-82 years (mean age 44.65 years and median 46.0 years). Fifty one patients (62.9%) were less than 50 years of age and only two patients aged over 70 years. Largest group of patients (20) were in the age group 41-50 years. (Fig 1)

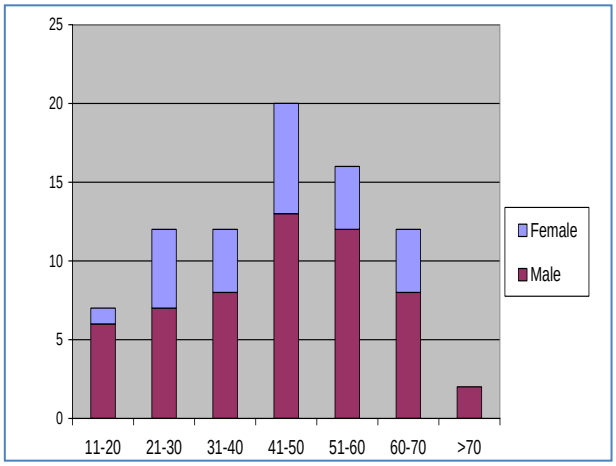

Fig. 1: Study patients: age and sex

Regarding the dietary habit, 54 patients (66.6%) were lacto-vegetarian, most of them consumed less than 500 ml of milk or other dairy products every day. Twenty-seven patients (33.3%) were non-vegetarian. Most of the non-vegetarian patients did not have daily intake of eggs, meat or fish in the diet. Nine patients consumed alcohol (not regularly) and four were smokers.

Duration of illness in most of the patients ranged from 1 week to 8 months. Three patients had duration of illness over one and half years. Many patients had been to other hospitals and taken some form of treatment and some of them affirmed intake of vitamins and general nutritional supplements. Precise details of treatment taken and records were not available in most of the cases.

Neurological examination findings:

Detailed neurological examination was carried out in all patients. Abnormalities of muscle tone, reflexes, loss of vibration and position sense were the commonest findings. Most common finding in neurological examination was impaired vibratory sensation (50.7%), followed by reduced muscle strength (50.6%). Abnormalities in deep tendon

reflexes both reduced and increased reflexes were present in thirty seven patients (45.6%). Increased tone (43.2%) was seen in more patients than reduced tone (39.5%). Gait abnormality was present in 28.5% of patients. Tests for touch, position, vibratory sensations and gait abnormalities could not be examined in 18 patients who were either disoriented or could not respond well to the commands and some of them were confined to bed.

Associated neuropsychiatric manifestations were present in 34.5% patients. Cognitive impairment was present in 29.6% patients. The neurological examination findings are presented in Table 2.

Table 1: Neurological examination findings (n=81)

Neurological finding	Number (n=81)	%
Impaired vibratory sensation *	32	50.7
Impaired position sensation *	29	46.0
Reduced reflexes	37	45.6
Increased reflexes	37	45.6
Reduced muscle strength	41	50.6
Increased Tone	35	43.2
Reduced Tone	32	39.5
Normal Tone	14	17.2
Abnormal gait *	18	28.5
Cognitive impairment	24	29.6
Neuropychiatric manifestations	27	33.3
Cranial nerve involvement	10	12.3

*Neurological findings tested in 63 patients

Hematological findings in patients of neurological syndromes:

Study parameters included Hemoglobin, Hematocrit, RBC count, Total leucocyte count, absolute counts for neutrophils, lymphocytes, eosinophils, basophils and monocytes along with red cell indices including MCV, MCH, MCHC, RDW and reticulocyte counts on Sysmex XT 2000 i analyzer.

Table 2: Hematological findings in patients of neurological syndromes:

Hematological findings	Mean (SD)	Median	Range (Min-Max)
Hemoglobin (gm/dl)	11.7 (2.37)	11.7	6.1-17.6
Hematocrit (%)	36.5 (7.0)	36.5	19.7-54.2
RBC count ($x10^6$/µL)	3.7 (0.79)	3.6	1.96-5.46
MCV (Fl)	99.4 (1.23)	98.4	74.3-131.8
MCH (Pg)	31.3 (4.89)	30.5	19.1-43.5
MCHC (g/l)	31.4 (1.87)	31.6	24.7-37.9
RDW (Fl)	55.1 (1.08)	52.5	39.9-86.1
TLC ($x10^3$/ µL)	8.3 (3.80)	7.8	1.7-23.7
PLT ($x10^3$/ µL)	212 (9.5)	191	73.0-578.0

Hematological abnormalities in study patients (n=81)

Abnormalities of peripheral blood were present in majority of patients. The most common peripheral blood smear findings present in this study was anemia, present in 48 patients (59.2%). Hemoglobin ranged from 6.1 to 17.6 g/dl. Anemia was defined as hemoglobin of <13 g/dl in males and hemoglobin of <12 g/dl in female patients. Hypersegmented neutrophils were present in 43patients (53%).

Macrocytosis was defined as MCV ≥100 fl, was present in 38 patients (46.9%), Increased MCH was present in 35.8% patients. Reduced MCV (<82Fl) and MCH were present in 6 patients (7.4%) and in 12 (14.8%) patients respectively. Red cell distribution width ((RDW) ranged from 39.9-86.1. RDW was increased in 46 patients (56.7%). Reticulocyte counts ranged from 0.2 to 5%. Reduced reticulocytes were present in eight patients (9.8%), in others the reticulocyte count was within normal range.

Thrombocytopenia (platelet counts <$150x10^3$/µl) was present in 18 (22.2%) patients. Most of these patients had mild (15) to moderate (3) degree of thrombocytopenia. Abnormalities of leucocyte counts were found in 19(23.4%) patients. Reduced leucocyte count was present in seven patients (8.6%). Increased total leucocyte counts were present in 12(14.8%) of cases. Pancytopenia was found in three patients (3.7%). Hematological abnormalities are presented in Table-6.

Table 3: Hematological abnormalities in study patients.

Hematological abnormality	Number of patients	%
Anemia (Hb M<13gm/dl, F<12gm/dl)	48	59.2
Mean corpuscular volume (≥100 Fl)	38	46.9
Hypersegmented neutrophils	43	53.0
Reduced reticulocytes (<0.5%)	8	9.8
Pancytopenia	3	3.7
Leucopenia (TLC <$4x10^3$/µl)	7	8.6
Thrombocytopenia (Platelets <$150x10^3$/µl	18	22.2

Bone marrow examination findings in neurological syndromes:

Bone marrow aspiration was done in all patients for presence of megaloblastic changes in smears in these suspected cases of nutritional neurological syndromes. Normal cellularity was present in 50.6% patients and in 43.2% patients marrow was hypercellular. Most common finding on bone marrow examination was reversal of myeloid and erythroid ratio (M:E ratio) and was present in more than three-fourth of patients (76.5%). Megaloblastic changes in erythroid cells were present in 42 patients (51.8%). Morphological findings in the erythroid lineage included presence of erythroid hyperplasia, increased number of proerythroblasts, increased cell size, nuclear cytoplasmic asynchrony, nuclear chromatin attenuation, abnormal mitosis, and evidence of maturational abnormalities. Most of the patients had mild megaloblastic changes and no patient showed severe megaloblastic changes. Normoblastic erythropoiesis was present in 39 (48.1%) patients.

Morphological findings suggestive of megaloblastic dysmaturation were commonly seen in erythroid precursors in the bone marrow and were present in 40 patients (49.3%). In some patients changes in the myeloid series were more pronounced than that in the erythroid precursors. Abnormal morphological findings included presence of giant myelocytes, metamyelocytes, hypersegmented forms and in some cases abnormalities of granulation. Increase in myeloblasts and significant dysplastic changes were not observed. Megakaryocytes were increased in 15 patients (18.5%). Megakaryocyte morphology was normal in most of the cases and in only few cases abnormal megakaryocyte morphology was present, including presence of separated nuclei and some degree of nuclear chromatin attenuation. In the aspirate smears micromegkaryocytes were not seen.

Table 4: Bone marrow examination findings in study patients

Bone marrow findings	Number of patients	%
Normal cellularity	41	50.6
Increased cellularity	35	43.2
Reversed M:E ratio	62	76.5
Megaloblastic erythropoiesis	42	51.8
Megaloblastic changes in myeloid series	40	49.3
Normoblastic erythropoiesis	39	48.1
Increased Iron stores	16	19.7
Reduced Iron stores	18	22.2
Increased megakaryocytes	15	18.5

Biochemical findings in neurological syndromes: Serum routine biochemistry analyses including serum bilirubin, lactate dehydroenase, serum creatinine and serum alanine were done on RX Imola Clinical Chemistry analyzer. Serum bilirubin ranged from 0.3 to 4.9 mg/dl, in most of the cases it was due to mild indirect hyperbilirubinemia. Serum LDH ranged from 90-1250 U/L. There were no significant renal function abnormalities in the study patients.

Table 5: Biochemical findings in patients

Biochemical findings	Mean (SD)	Median	Range (Min-Max)
Serum bilirubin (mg/dl)	0.94 (0.71)	0.75	0.34-4.9
SGPT (U/L)	43.7 (4.8)	25.0	6.0-316
LDH (U/L)	624 (3.3)	495.5	90-1250
Serum creatinine (mg/dl)	1.01 (0.34)	0.98	0.49-2.5
Serum vitamin B12(pg/ml)	737.1 (6.7)	478.0	29.0-2355
Serum copper (µg/ml)	1.33 (0.55)	1.28	0.51-2.53
Serum zinc (µg/dl)	0.77 (0.27)	0.69	0.28-1.32

Table 6: Biochemical abnormalities in study patients

Biochemical abnormality	Number of patients	%
Increased bilirubin	19	23.5
Increased LDH (n=46)*	26	56.5
Increased creatinine	5	6.1
Increased SGPT	26	32.1

Biochemical abnormalities in neurological syndromes:

Biochemical tests revealed increased serum LDH in 56.5% of patients. Increased liver enzymes were found in 32.1% and increased serum bilirubin in 23.5% patients

Vitamin B12 status in patients with neurological syndromes:

Vitamin B12 was assessed by a solid phase, competitive chemiluminiscent enzyme immunoassay. Vitamin B12 deficiency was defined as serum vitamin B12 level less than 200 pg/ml. Out of eighty one patients, twenty eight patients (34.5%) were found to be Vitamin B12 deficient. Twenty six patients (32.0%) had serum vitamin level within normal range (200-950 pg/ml) and twenty seven patients (33.3%) had serum vitamin B12 level in higher range (more than 950 pg/ml).

Five patients had vitamin B12 level <100pg/ml. Mean levels in deficient group were 133.8 (4.14) pg/nl, median 150 pg/ml and ranged from 29.9-177 pg/ml. In the normal B12 group, mean levels were 459.3(1.88) pg/ml, median 424 pg/ml and ranged from 211-870 pg/ml.

In the high B12 group, mean levels were 1455(5.0) pg/ml, median 1200 pg/ml and ranged from 955-2355 pg/ml. In several studies Vitamin B12 levels upto 300 pg/ml are taken as borderline. In our study five patients had Vitamin B12 levels between 200-300 pg/ml.

Table 7: Status of vitamin B12 in neurological syndromes

Vitamin B12 (n=81) & Folate status (n=38)	Number of patients (%)
Vitamin B12 deficient patients	28 (34.5)
Vitamin B12 non- deficient patients	26 (32.0)
Patients with high vitamin B12 levels	27 (33.3)

Patient characteristics and clinical presentation

The study included 81 patients that presented with various neurological syndromes including features suggestive of myeloneuropathy (32) myelopathy (11), and neuropathy (38) with or without neuropsychiatric manifestations. Duration of illness of these groups was that in myeloneuropathy 1 week to 10 months (one patient had h/o illness 5 years), in myelopathy 1 week to 2 years and in neuropathy 1 week to 18 months.

Males to female ratio for all study patients were 2.1:1. In patients with myeloneuropathy it was 1.4:1, for myelopathy and neuropathy the M:F ratio was 2.6:1 and 2.8:1 respectively. So in females patient's myeloneuropathy was the more common presentation, seen in 50% of the patients. In male patients neuropathy was seen in higher numbers.

Table 8: Clinical characteristics of patients in neurological syndromes.

Neurological syndrome	Myeloneuropathy	Myelopathy	Neuropathy
Number of patients	32	11	38
Mean age	42.6 (1.6)	45.5(2.08)	46.1 (1.41)
Median age	42.5	48	48.5
Age range	14-69	17-81	19-82
Male: female ratio	1.4:1	2.6:1	2.8:1
Veg: non-vegetarians	24/8	5/6	25/13

The proportion of vegetarians patients was 2:1 compared with the patients with nonvegetarians food habits. In myloneuropathy vegetarians were 3 times more than non vegetarians. In mylopathy both vegetarians and nonvagetrians were approximate in equal number. In neuropathy vegetarians were near about 2 times more than nonvegetarians.

Laboratory findings in myeloneuropathy, myelopathy and neuropathy subgroups

In all patients anemia was present in 48 patients (59.2%). In the neuropathy group anemia was present in 73.6% of patients, which was much higher compared to the myelopathy and myeloneuropathy groups.In patients with neuropathy anemia was present in more patients as compared to myeloneuropathy, the difference was statistically significant ($\chi 2=4.2$ and $p<0.04$).

Reduced iron stores were also present in higher proportion in neuropathy group. Patients in the myelopathy group had higher prevalence of macrocytosis, hypersegmented neutrophils, thrombocytopenia, and megaloblastic changes compared to those in myeloneuropathy and neuropathy groups.

Table 9: Laboratory finding in various neurological syndromes

Laboratory findings	Myeloneuropathy (32)	Myelopathy (11)	Neuropathy (38)
Anemia	15 (46.8)	5 (45.4)	28 (73.6)
MCV (≥ 100fl)	16 (50)	6 (54.5)	15 (39.4)
Hypersegmented neutrophils	16 (50)	7 (63.6)	20 (52.6)
Thrombocytopenia	6 (18.7)	4 (36.3)	8 (21.0)
Increased cellularity	11 (34.3)	5 (45.4)	19 (50)
Reversed M:E ratio	23 (71.8)	10 (90.9)	29 (76.3)
Megaloblastic erythropoiesis	17 (53.1)	6 (54.5)	19 (50)
Reduced Vitamin B12	10(31.2)	5(45.4)	13(34.2)
Increased bilirubin	7 (21.8)	2(18.1)	10 (26.3)
Increased LDH (n=46)*	12 (52.1)	4 (80)	10 (55.5)
Increased SGPT	11 (34.3)	3 (27.2)	12 (31.5)

In patients with myeloneuropathy, 31.2 % had Vitamin B12 deficiency, 37.5% had normal B12 levels and 31.2 % had high B12. Vitamin B12 deficiency was seen in 10 patients of myeloneuropathy (31.2%), 5 patients of myelopathy (45.4%) and 13 patients of neuropathy (34.2%).

Discussion:

Based on history, symptoms, clinical presentation and neurological examination findings, the presenting neurological syndrome were classified as myeloneuropathy, myelopathy or neuropathy.

- Based on the clinical presentation and neurological examination findings the patients were classified as having myelopathy (11 patients), myeloneuropathy (32 patients) or neuropathy (38 patients).

- The most common peripheral blood smear findings present in this study was anemia, present in 48 patients (59.2%) followed by hyper segmented neutrophils in 43 patients (53%) and macrocytosis in 38 patients (46.9%).

- Bone marrow aspiration was done in all patients for presence of megaloblastic changes. Most common finding on bone marrow examination was reversal of myeloid and erythroid ratio (M:E ratio), presented in 76.5% patients, followed by megaloblastic changes in erythroid cells in 42 patients (51.8%). Most of the patients had mild to moderate megaloblastic changes.

- We found increased lactate dehydrogenase in 56.5% patients, hyperbilirubinemia in 23.5% patients, increased serum alanine transferase in 32.1% patients and increased serum creatinine in 6.1% patients.

- In patients having hyper segmented neutrophils, some neurological features as weakness, memory loss and seizures were present more often and the association was statistically significant ($\chi 2=4.0$, $p<0.04$, $\chi 2=9.2$, $p<0.002$ and $\chi 2-=4.7$ $p<0.02$, respectively).

- We had follow-up hematological assessment in 53 patients (65.4%) who attended neurological OPD for subsequent neurological consultation and management for a period of three months to one year. Treatment response was assessed by neurological examination, hematological and neurophysiology studies. Bone marrow examination was not done during follow-up and only peripheral blood was examined.

- Out of these 53 patients, 38 patients (71.6%) showed good hematological response with rise in hemoglobin levels and eight patients (15%) converted from anemic to non-anemic status. In 28 patients (52.8%) fall in MCV was observed and 15 patients (28.3%) showed reduction of MCV from high to normal range. In 18 patients (33.9%) having pre-treatment hyper segmented neutrophils, the peripheral blood smears during follow up showed normal segmented neutrophils. In nine patients (16.9%), rise in

reticulocyte count was observed from ≤0.5% to more than 5%.

- Eight patients of vitamin B12 deficient group achieved completely normalized hematological profile although some degree of residual neurological impairment was still present. Many patients included in this study continue to be in follow-up with the Neurology department.

Hematological response on follow-up

Early therapy is instituted for patients with vitamin B12 deficiency neurological syndrome. In responders to the therapy reticulocytosis begins early and peaks within a weak hemoglobin concentration rises within two weeks of therapy and normalises within three months. Hyper segmentation may persist for 2-4 weeks.

At our centre as per protocol, daily 1000 microgram vitamin B12 by intramuscular injections was given for 10 days followed by 1000 microgram per week for one month than one injection monthly. Thereafter, the patients were advised regular follow-up in Neurology OPD. Treatment response was assessed by neurological examination, hematological tests and neurophysiology studies. Bone marrow examination was not done during follow-up and only peripheral blood examination was done.

We had follow-up hematological assessment in 53 patients (65.4%) who attended neurological OPD for subsequent neurological consultation and management for a period of three months to one year. Nearly two third of the patients showed complete neurological function recovery and one third showed partial functional improvement. Improvement was particularly seen in lower limb power, vibratory and position sense. However, abnormality of reflexes persisted.

Out of these 53 patients, 38 patients (71.6%) showed good hematological response with rise in hemoglobin levels from 1 to 4 g/dl in most of them and eight patients (15%) converted from anemic to non-anemic status. In 28 patients (52.8%) fall in MCV was observed and 15 patients (28.3%) showed reduction of MCV from high to normal range. In 18 patients (33.9%) with hypersegentation of neutrophils in peripheral blood smears showed conversion to normal segmented neutrophils. In nine patients (16.9%) rise in reticulocyte count was observed from ≤0.5% to 5%. Eight patients of vitamin B12 deficient group achieved completely normalized hematological profile but some degree of residual neurological impairment was still present. Many of the patients included in this study continue to be in follow-up with the Neurology department.

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