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A CLINICAL STUDY ON THE MANAGEMENT OF VITAMIN B12 (COBALAMIN) DEFICIENCY POLYNEUROPATHY WITH AMALAKI RASAYANA

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ABSTRACT
Vitamin B_{12} is an essential vitamin that has a important role in the functioning of peripheral and central nervous system. Adequate B_{12} status is crucial for normal neuro-development as its deficiency affects the nervous system, resulting in demyelination of peripheral and central neurons, which is generally considered to be the mechanism underlying the classic neuropathy of B_{12} deficiency. The reasonable way to understand cobalamin deficiency polyneuropathy comes around a subcategory of *Vata vyadhi. Rasayana* due to its antioxidant & adaptogenic qualities is an excellent remedy to correct these abnormalities. **Materials and Methods:** Twenty cases of Vitamin B_{12} deficiency polyneuropathy were diagnosed. Ten cases were administered with *Amalaki Rasayana* & ten cases were administered with Methycobalamin. The outcome was assessed on ACTG peripheral neuropathy symptom score. **Results:** *Amalaki rasayana* shows significant improvement in pain, location of pain , numbness, location of numbness, symptoms grading score & serum B_{12} values. **Discussion:** The action of the drug is due to the acting properties of the drug viz, *Rasayana* as well as *Vatahara* & *Srotoshodhana* and giving significant improvements in subjective and objective parameters of assessment. **Conclusion:** The study confirms that *Amalaki rasayana* selected for the study is effective in treating the disease.

KEY WORDS: *Amalaki Rasayana*, vitamin B12 deficiency, neuropathy.

INTRODUCTION

Nutritional deficiency is from various combinations of starvation, abnormal assimilation of the diet, the stress response of illness, and abnormal nutrient metabolism. Intestinal mucosal malabsorption (as in celiac disease) is commonly associated with additional deficiencies of other water soluble Vitamins. Vitamin B_{12}, also called cobalamin, is a water-soluble Vitamin that has a key role in` the normal functioning of the brain and nervous system, and the formation of red blood cells. Some research states that certain non-animal products possibly can be a natural source of Vitamin B_{12} because of bacterial symbiosis.[1]

Vitamin B_{12} consists of a class of chemically related compounds (Vitamers), all of which show pharmacological activity. It contains the biochemically rare element cobalt (chemical symbol **Co**) positioned in the center of a planar tetra-pyrrole ring called a corrin ring.[2]

The total amount of Vitamin B_{12} stored in the body is between two and five milligrams in adults. Approximately 50% is stored in the liver, but approximately 0.1% is lost each day, due to secretions into the gut not all of the Vitamin in the gut is reabsorbed. While bile is the main vehicle for B_{12} excretion, most of the B_{12} secreted in bile is recycled via enterohepatic circulation.[3]

Vitamin B_{12} deficiency is a common but under-recognized, yet easily treatable disorder in older adults. Causes of deficiency include failure to separate Vitamin B_{12} from food protein, inadequate ingestion, absorption, utilization, and storage as well as drug-food interactions leading to malabsorption and metabolic inactivation.[4]

Generally in all ages the causes of cobalamin defiency are infections like H.pylori, Giardia lamblia, fish tapeworm; malabsorption & medical conditions like Gastric resection, inflammation of small intestine, Crohn disease.[5]

It has been suggested that serum cobalamin <148 pmol/L (200 ng/L) would be sensitive enough to diagnose 97% of patients with Vitamin B_{12} deficiency.[6] The clinical picture is the most important factor in assessing the significance of test results assessing cobalamin status

because there is no 'gold standard' test to define deficiency.[7]

Vitamin B_{12} deficiency can occur even in people who consume meat, poultry, and fish. An estimated many of the patients with neurological signs do not manifest anaemia.[8]

Neurological manifestations occur due to Vitamin B_{12} deficiency. There is demyelation of peripheral nerves, posterior nerves, posterior & lateral columns of spinal cord & brain. Patient presents with paraesthesia & numbness of extremities, weakness & sensory ataxia. Involvement of brain results in irritability, dementia or frank psychosis.[9]

Ayurveda considers neurological disorders to be the result of a *Vata* disorder. The vitiation of *Vata dosha* causes an imbalance and disharmony in the human system that leads to neurological disorders. *Ayurvedic* treatments for neurological disorders will aim to rectify this *Vata* imbalance and bring the *Vata dosha* in harmony with *pitta* and *Kapha dosha* so as to eliminate every type of disease in an individual.[10,11]

MATERIAL AND METHODS

Aims and Objectives

1. To clinically evaluate the efficacy of *Amalaki Rasayana* in the management of Vitamin B12 (cobalamin) deficiency polyneuropathy based on criteria of assessment.
2. To clinically evaluate the effect of oral Methycobalamin in the management of Vitamin B12 (cobalamin) deficiency polyneuropathy based on criteria of assessment.
3. To compare the effect of *Amalaki Rasayana* & Methycobalamin in the management of Vitamin B12 (cobalamin) deficiency polyneuropathy based on criteria of assessment.

Selection of patients

Total 20 Patients of Vitamin B_{12} deficiency polyneuropathy were selected from the O.P.D. Dept. of Kayachikitsa, Parul Ayurveda hospital, on the basis of inclusion and exclusion criteria, depending on the detailed clinical history, physical examination and other necessary / desired investigations and irrespective of their gender, caste or creed.

Study Design: Randomized clinical Trial – Flip coin method.

Inclusion Criteria

- People with age group of 20-60 years
- Clinical features of vitamin B12 (cobalamin) deficiency polyneuropathy like numbness, burning sensation, pricking pain etc.
- Low levels of serum B12 ≤ 150 pmol/L (200 pg/mL).[12]

Exclusion Criteria

- B12 deficiency due to any secondary systemic illness/disease like Malabsorption syndrome, crohn's disease
- Uncontrolled systemic diseases like Diabetes Mellitus & Hypertension

Patients fulfilled the criteria for diagnosis were included in the study and subjected to thorough interrogation and physical examination.

Laboratory Investigations: Serum B12.

Study Plan

	Group A	**Group B**
Drug	*Amalaki Rasayana*	Methycobalamin
Form	Tablet	Tablet
Dose	3 tablets of 500 mg each	500mcg
Frequency	Twice in a day before meal	Once in a day after meal
Duration	8 weeks	8 weeks
Follow up	4 weeks	4 weeks

Criteria for assessment

- The assessment of the trial was done on the basis of following parameters.

Subjective: The subjective assessment was done on the basis of improvement in ACTG peripheral neuropathy symptoms before and at the end of the trial.

Objective: The objective assessment was done on the basis of changes in serum B_{12} values before and at the end of the trial.

Preparation of the drug[13]

Firstly fresh fruits of *amala* were taken & washed with water to remove physical impurities. Then fruits were boiled upto the consistency to mash the fruits easily. Seeds were removed & fruit pulp were mashed to make a paste form. Sodium benzoate was added to it as a preservative. This paste was processed with fresh *amala swaras* in edge runner daily for 14 days. After 14 days completion of *swaras* process, it was dried in oven & was converted into tablet form of 500 mg each.

RESULT

Effect of therapy on Pain, aching & burning (Paired t test)

	Group A (*Amalaki Rasayana)*		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	9.40	8.10	7.20	4.40
t value	2.32		4.610	
p value	0.045		0.007	
Remarks	Significant		Highly Significant	

Mann-whitney test for comparison between the groups on Pain,aching & burning

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Pain, aching & burning after treatment	Group A	8.70	87.0	-1.47	0.14	NS
	Group B	12.30	123.0			

Effect of therapy on Pins & needles (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	10.20	9.50	7.50	5.00
t value	1.48		3.55	
p value	0.17		.006	
Remarks	Not Significant		Highly Significant	

Mann-whitney test for comparison between the groups on Pins & needle

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Pins & needle after treatment	Group A	8.20	82.0	-1.97	0.48	S
	Group B	12.80	128.0			

Effect of therapy on "Numbness" (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	6.40	4.60	5.30	1.40
t value	5.04		7.73	
p value	0.001		0.000	
Remarks	Highly Significant		Highly Significant	

Mann-whitney test for comparison between the groups on "Numbness"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Numbness after treatment	Group A	6.55	65.50	-3.05	.002	S
	Group B	14.45	144.50			

Effect of therapy on "Symptoms grading score " (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	2.40	1.60	2.30	0.90
t value	6.00		8.57	
p value	0.000		0.000	
Remarks	Highly Significant		Highly Significant	

Mann-whitney test for comparison between the groups on "Symptoms grading"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Symptoms grading after treatment	Group A	7.90	79.0	2.43	0.01	S
	Group B	13.10	131.0			

Effect of therapy on "Location of pain" (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	1.50	0.40	2.20	0.80
t value	2.28		3.50	
p value	0.048		0.007	
Remarks	Significant		Highly Significant	

Mann-whitney test for comparison between the groups on "Location of pain"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Location of pain after treatment	Group A	9.80	98.0	-0.57	0.56	NS
	Group B	11.20	112.0			

Effect of therapy on "Location of pins & needles " (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	0.70	0.60	2.0	0.50
t value	1.00		2.76	
p value	0.34		0.022	
Remarks	Not Significant		Significant	

Mann-whitney test for comparison between the groups on "Location of pins & needles"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Location of pins & needles after treatment	Group A	8.25	82.50	-2.09	0.03	S
	Group B	12.75	127.50			

Effect of therapy on "Location of Numbness " (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	3.30	2.90	3.20	1.00
t value	2.44		4.71	
p value	0.037		0.001	
Remarks	Significant		Highly Significant	

Mann-whitney test for comparison between the groups on "Location of numbness"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Location of numbness after treatment	Group A	7.10	71.0	-2.68	.007	S
	Group B	13.90	139.0			

Effect of therapy on "Vibration perception score" (Paired t test)

	Group A (*Amalaki Rasayana*)		Group B (Methycobalamin)	
	BT	AT	BT	AT
Mean	0.90	0.60	0.70	0.10
t value	1.96		3.67	
p value	0.081		0.005	
Remarks	Not Significant		Highly Significant	

Mann-whitney test for comparison between the groups on "Vibration perception score"

Parameter	Group	Mean ranks	Sum of ranks	Z	P value	Remarks
Location of vibration perception score after treatment	Group A	9.00	90.0	-1.31	0.18	NS
	Group B	12.00	120.0			

Effect of therapy on Serum B_{12} values (t test)

	Group A (*Amalaki Rasayana*)	
	BT	AT
Mean	141.2	182.30
Mean Difference	41.10	
t value	4.02	
p value	0.003	
Remarks	Significant	

DISCUSSION

Charaka's view in approach of disease understanding

Ayurveda is an experiential science that stands largely on clinical presentations rather than investigations for construction of a supporting knowledge regarding etiopathology of a disease. The research question- Vitamin B_{12} deficiency Polyneuropathy should be understood through the *Charaka's* dictum without deviating from the principles of *Ayurveda*. The methodology of understanding an unknown disease is described in *Charaka Samhita* based on *Aptopadesha*.[14] *Acharya* mentions that diseases are innumerable, which differ from each other by its nature, place where it manifests, symptoms, etiology and minute differences in combinations and permutations of *dosha* and *dushyas* during the pathogenesis.[15] Altogether they are *Prakopana*, *Yoni*, *Utthana*, *Atmana*, *Adhisthana*, *Vedana*, *Samsthana*, *Shabda*, *Sparsha*, *Rupa*, *Rasa*, *Gandha*,

Upadrava, *Vridhi-Sthana-Kshaya*, *Udarka*, *Nama*, *Yoga* and *Pratikarartha Pravritti* and *Nivritti.*[16]

***Vataprakopa*:** The *dushti* of *Vata*; due to *Swanidana* or *Vyadhi karshan* is the first factor in the pathology of polyneuropathy. The *vata prakopa* may get by its own *nidana* can produce symptoms. While on the other hand, *vyadhikarshan* which is leading to *dhatu kshaya* can lead to *Vata prakopa*.

***Oja Kshaya*:** *Oja kshaya* is due to the result of impaired *dhatuposhana*-as the *oja* is the *sara* of all *dhatus*.

***Rasayani Daurbalya*:** The *Vyana Vata prakopa* secondary to *Avarana* by *Kapha bhavas* are specially noticed by the higher incidence of involvement of distal parts especially lower limbs due to the *daurbalya* of *rasayanis*[17] in that area. The incidences of neuropathy depends on length as the axons which are at the distal end get impacted firstly because they are far away from the spinal cord.

***Indriya Pradosha*[18]:** The clinical impacts of *indriyas* are also noteworthy looking in to the late complications on nerves. The *Sparshanendriya* is the *sthana* of *Vata*[19] and *Sparshana* is *indriyartha*. *Vata* is inseperably related to the functioning of Skin and tactile perception; hence *twak* gets the effect of the *dushti* of its *ashrayee dosha* changes. The *twak* and *rasa* are the *dushyas* in the pathology, undeniably get the impact of the disease process. Chakrapani clarifies that *Indriya upatapa*[20] is the hampered & reduced functioning (*kinchit vaikalyam*) of *Indriya* due to the *ashraya* of *dushta doshas* that are residing in it. The reduction in touch & pain perception is the consequent of disease condition on *Twak indriya*.

***Avarana*:** The *Vata* may get obstructed i.e. *Avarana* by the *vridha dushya's* and produces the signs & symptoms of *Kapha* (*ama*/ *avaraka*) or *vatakarma kshaya*. The *aggravated Vata* may get *Avarana* by normal *dushyas* which produces symptomatology. The third factor is that the *vriddha dushyas* leading to hampering of gati of normal *Vata* that further leading to clinical manifestations. The *Avarana* process is understood by *roopavriddhi*, *roopahani* or *roopantara* of *Vata* in Vitamin B_{12} deficiency polyneuropathy. The *lakshanas* of the factors which leads to *Vata Avarana* is presented as clinical manifestations.

Discussion on *Amalaki Rasayana*

Vitamin B_{12} deficiency polyneuropathy is characterized due to *Vata dushti*. The pathogenesis is complex i.e. *dhatukshaya* produced by *Vata prakopa* either due to *swa-nidana* or by the *Avarana* of *Kapha*/ *ama*/ *kaphavargeeya dushyas*, *rasayani daurbalya* that contributes to the pathology. The lack of cardinal feature and the varying clinical characteristics suggests the role of *Avarana* of *Vata*. The *Chikitsa sutra* of *Avarana*[21] clearly mentioned about the ideal drug which should be *Anabhishyandi* as well as *snigdha* and *srotoshodhana* property. The parameters in pathogenesis like *Srotorodha* which is caused to the microcirculation will be rectified with the administration of drug along with prevention of further accumulation of *dosha* and clearing the path for normal *gati* of *Vata* for its proper functioning. The drug action should not oppose the functions of *Kapha* and *Pitta*, at the same time inducing *Vatanulomana*. The ideal drug should be *Rasayana* as the *Dhatushaithilya* is the main cause in the pathogenesis of Vitamin B_{12} deficiency Polyneuropathy.

The selected polyherbal formulation- *Amalaki Rasayana* was utilized as *naimittika Rasayana* in the study.

The drug *Amalaki Rasayana* tablets were administered which is easy to dispense, has a better shelf life. The advantage of tablets is that the measured dose is followed by the included subjects. Three tablets of 500mg were administered twice daily for 8 weeks with warm water.

Discussion on Methodology

The salient features of the study were;

- This clinical study is registered in Clinical trial registry of India. The summary of the trial protocol can be accessed anywhere from the CTRI website. Thus making it more transparent.
- Written informed consent was obtained prior to the registration of each subject in accordance with the WHO ERC guidelines.[22]
- Validated, reproducible, sensitive and specific criteria of outcome assessment including subjective and objective parameters were used in the study.

Discussion on Observations

A total of 25 subjects were taken up with intention to treat and were allocated in to two groups; Group A (*Amalaki Rasayana* Group) and Group B (Methycobalamin Group) which consisted of 10 and 15 subjects respectively. 5 Subjects in Group B lost to follow up without citing any reason. The assessment of therapies was based on 20 cases who completed the follow up.

Age: Maximum number of subjects (70%) belonged to 20-30 years of age group. The incidence of Vitamin B_{12} deficiency polyneuropathy depends on the lifestyle. As youngsters are having irregular lifestyle which may lead to many lifestyle disorders.

Gender: Maximum numbers of female subjects were recruited for the study, i.e. 70% (14) females as females are more prone to faulty dietary habits.

Economic status: Majority of included subjects belonged to middle income group i.e. 11 (55%), lower middle i.e. 5 (25%) & poor i.e. 2 (10%). This is due to the settings of the study which caters to the need of underprivileged in the society. Low to middle socioeconomic status contributes to the development of complications of Vitamin B_{12} deficiency.[23]

Religion: Majority of the included subjects 19 (95%) were Hindus which shows the geographical predominance of the faith in the subjects.

Educational status: The incidence was observed mostly in people who are educated up to Graduation 10 (50%) & 4 (20%) were educated upto high school. This probably indicates the general level of education in subjects visiting study centre.

Chief Complaints and duration

Maximum number of subjects complained of Numbness as the major negative sensory complaint i.e., 19 (95%), pain as positive sensory complaint i.e. 12 (60%), Pins & needle as positive sensory complaint i.e. 8 (40%), Burning sensation as a positive sensory compliant i.e. 3 (15%), Aching as a primary sensory complaint i.e. 2 (10%). In polyneuropathy the clinical feature depends upon the type of nerve fiber involved[24]; large fiber neuropathy causes sensory deficits for vibration and touch, whereas small fiber involvement causes thermal (hot, cold) perception and allodynia.

Maximum number of patients i.e. 15 (75%) were having symptoms duration less than 6 months.

Dietary habits: All subjects i.e. 20 (100%) were accustomed to vegetarian food. This feature probably has role in the disease onset as dietry intake has a important role in vitamin B_{12} deficiency. As main source of vitamin B_{12} is animal origin, while milk & its products are having low vitamin B_{12} status. So the prevalence rate of vitamin B_{12} is more seen in vegetarians as compare to non-vegetarians.[25]

Dominant *Rasa* in diet: even though all the included subjects were having all *rasas* in their diet, Maximum 14 (70%) were habituated for *Madhura rasa*, followed by *katu rasa* (20%). Excessive use *madhura* rasa leads to *agnimandya* which further leads to *dhatukshaya* & *vata vyadhi.*[26]

Dominant *Guna* in Diet: Maximum number of included subjects were accustomed to *Rooksha* & *ushna Bhojana*; which further leads to *vata vyadhi.*

Tobacco abuse: Only one of the included subjects were addicted to tobacco abuse either chewing or smoking. Its established fact that tobacco is a risk factor that fastens up nerve damage.[27]

Discussion on Effect of therapy

Effect of therapy on ACTG peripheral neuropathy symptom score: It was used in the study to setup the presence and to notice the changes to provide a clinical correlation for the outcome measures. The *Amalaki Rasayana* (Trial drug) and Methylcobalamin (control drug), both provided improvements in Actg peripheral neuropathy symptom score which was statistically significant. There was statistically significant improvement in Serum B_{12} values after treatment with *Amalaki rasayana* when compared with mean values before treatment.

There were total 8 assessment points that has been evaluated to notice the effect of drug on ACTG peripheral neuropathy symptom score For comparing between the group, Mann whitney test was used.

Between group analysis showed that there was no significant difference in mean values of pain, aching & burning, location of pain, vibration perception score of group A (*Amalaki Rasayana*) & group B (Methycobalamin). Both the control drug & trial drug may have provided relief in polyneuropathy by restoration of the endothelial complex and renewing distal perfusion. The significant result in pain from *amalaki* may be seen due to phenolic compounds for amelioration of pain due to their modulatory action of free radicals.[28,29] According to a research work done in Lebanon-New Hampshire, the result in recovery of neuropathic pain is faster & more efficient as comparable to numbness & paresthesia.[30] It works as analgesic[31] reducing symptoms of neuropathy.

Amalaki is also a proven anti-oxidant[32] reducing the oxidative stress over the peripheral nerves thus it may provide relief in numbness & paraesthesia. *Amalaki* has a significant ulcer protective as well as healing effects which might be due to its effects both on offensive and defensive mucosal factors.[33]

Effect of therapy on serum B_{12} values

Eighty percent of cobalamin is stored in liver, which is further transported to tissues. Inflammation and oxidative stress contribute to liver injury & *amalaki* which is rich in vitamin C, gallic acid, flavonoids, and tannins, protects against hepatoxicity. *Amalaki* supplementation is known to heal liver injury via its antioxidant, ant-inflammation, anti-apoptosis, and anti-autophagy properties.[34] Thus *Amalaki rasayana* may have significant role in increasing serum B_{12} values by increasing its absorption in circulation as well as storage in the liver through dairy & fortificated food products. *Amalaki* is known to have affirmative role in the management of Neuropathy.

Both the groups showed efficacy in reducing the subjective symptoms of polyneuropathy. However, Group B provided better result as per the change observed in pre test post test means. On comparison between both groups, the result observed was denoting group B have more effect on ACTG peripheral Neuropathy symptom score.

Probable mode of action of *Amalaki Rasayana*

The trial drug *Amalaki Rasayana* contains *Amalaki* and *Bhavana* of *Amalaki swaras* in it. The probable mode of its action is discussed here under.

Amalaki is considered to pacify *vata dosha* by its *amlatwa* property thus giving relief in *Ruja*.

Supti is considered as *Kaphaja Nanatmaja vikara. Amalaki* is having *kashayatwa* & *rookshatwa* property which helps in reducing *supti* because of it *kaphahara* action. *Amalaki* is a well known *Rasayana* possessing *madhur*, *sheeta* property reduces *daha* associated with Polyneuropathy. There is *dhatwagni mandya* and *strotodushti*, both related to the onset of neuropathy & *amalaki* is known to have a positive role in *dhatwagni* & *strotoshodhan.*

Discussion on overall effects of therapy

The overall effect of therapy was calculated using the criteria of assessment in the interpretation of results.

This overall effect of therapy shows that Methycobalamin exerts better improvements in primary outcome measures in vitamin B_{12} deficiency polyneuropathy.

Amalaki rasayana shows significant improvement in pain, location of pain, numbness, location of numbness, symptoms grading score & serum B_{12} values while in symptom of pins & needles, vibration perception didn't showed significant result statistically. However insignificant result might have been obtained due to the less sample size. Ankle reflex score was normal in all the subjects, which further indicates exclusion of myelopathy. The action of the drugs are due to the acting properties of the drug viz, *Rasayana* as well as *Vatahara* & *Srotoshodhana* and giving significant improvements in subjective and objective parameters of assessment.

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