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Effect of Endurance Training on Homocysteine Level in Elderly with Megaloblastic Anemia

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Abstract: ***Background:*** Vitamin B12 deficiency causes megaloblastic anemia and it's a major contributor to hyper-homocysteinemia which have been linked to the risk of vascular disease, stroke, and dementia and are relatively common in elderly. ***Aim of Study:*** To investigate effect of endurance training program on homocysteine level in elderly patients with megaloblastic anemia. ***Methods:*** Sixty elderly patients suffered from megaloblastic anemia, participated in this study were selected randomly from out-patient clinic of internal medicine in Sers Ellyan hospital with age mean ± SD values in groups (A) and (B) were 68.23±2.74 and 67.83±2.19 years, respectively. The study design was pre-post study. Patients were assigned for 16 weeks protocol into two equal groups: Group (A) (study group): received selected endurance training 3 times a week for 16 weeks with controlled dietary vitamin B12 intake, while Group (B) (control group): received only B12 supplementation. The outcome measures were serum vitamins B12, total homocysteine (tHcy), mean corpuscle volume, 10MWT test and physical characteristic (weight and body mass index (BMI)) were evaluated at the baseline and the end of the study in both groups. ***Results:*** Pre- and post-treatment comparisons showed a statistically significant increase of the measured variables in the group A (serum B12 47.68%, 10MWT 40.86%) and significant decrease in MCV 15.62%, tHcy 28.93%. Group B showed a statist
(serum B12 134.76%, 10MWT 8.33% and significant decrease in MCV 17.97% and tHcy 37.31%). There were significantly higher change differences in the study group regarding dietary B12 and 10MWT compared to the control group (P<0.001 for all), a significantly lower change was observed in serum B12 and tHcy in the study group compared to the control group (P<0.001 for all) and no significant difference was detected between the two groups in MCV change (P=0.122). ***Conclusion:*** Endurance training program improved homocysteine and vitamin B12 biomarkers but not as conventional therapy and significantly improved gait speed.

Key Words: Endurance training, Homocysteine, Elderly, Megaloblastic anemia, B12.

1 Introduction

Cobalamin deficiency is more prevalent in elderly, especially between those above 65 years old, where vitamin B12 deficiency is common for about 10%-15% among them [1].
Cobalamin deficiency causes elevation of methylmalonic acid and homocysteine [2], compromised DNA formation, and hematologic abnormality like development of hyper-segmented neutrophils and megaloblastic anemia, a state where the bone marrow makes abnormally huge, atypical formation and incomplete RBCs named megaloblasts as a result of inefficient hematopoiesis [3].

Macrocytic anemia with elevated mean corpuscular volume (MCV), above 100 fL, it is the indicator of megaloblastic anemia, but leukopenia and thrombocytopenia also often exist [4].

Cobalamin insufficiency and B9 intake has been involved in homocysteine increase. [5]. In addition, serum total homocysteine is a specific indicator of B12 low values and elevates early in the progression of deficiency and continues to elevate more as the deficiency get worse [6].

Cobalamin deficiency in older age individuals varies from inability to absorb B12 and/or low nutritional ingestion due to various elderly-related comorbidities and related disorders [1]. Poor nutritional intake of cobalamin, inability to have cobalamin fortified products or cobalamin supplementation all triggers more the reduction in cobalamin. This could be an influential but modifiable cause of low cobalamin levels [7].

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In individuals having deficient low cobalamin status, elevated concentrations of methylmalonic acid and Hcy have been recognized to cause myelin degeneration (myelopathy) and, as a result, leads to peripheral and autonomic neuropathy [1].

Higher concentration of Hcy, called hyperhomocysteinemia (hHcy), is related to a greater possibility of neurovascular disorders, Alzheimer's, headache, growth defects or epilepsy [8], reduced muscular strength [9], slow gait speed and decreased physical abilities [10].

Aim of the study: This study aimed to evaluate the effect of endurance training program on homocysteine level in elderly patients with megaloblastic anemia.

2 Patient and Methods

The study was performed at Sers Ellyan hospital, Physical Therapy department outpatient clinic from August 2021 to April 2022.

Ethical consideration:

The study was approved from the Ethical Committee of Faculty of Physical Therapy, Cairo University. All patients were informed about the nature and purpose of the study, what would occur during the treatment sessions with extended opportunity to ask questions then signed an informed consent. Ethical consideration number: P.T.REC/012/001871.

A-Inclusion Criteria:

Sixty elderly patients of both sexes were enrolled in this study according to the following criteria:

Their age ranged from 65-75 years old, their body mass index ranged between (25 to 35 kg / m2). They were hyperhomocyteinemic (tHcy > 20 µmol/L), B12 deficient (B12 < 150 pmol/L) and examination of the blood smear showed anemia (MCV > 100 fL). They had neurologic symptoms in from of symmetric paresthesias, numbness, and impaired vibration and position sense leading to gait disturbances.

B-Exclusion Criteria:

Patients with a known history of uncontrolled hypertension, ischemic attacks, stroke, congestive heart failure, restrictive lung disease or obstructive lung disease, severe osteoporosis or severe osteoarthritis, dementia, suffering from malabsorption-related surgery or diseases, patients receiving B12 supplements and BMI > 35 excluded from the study.

Evaluation procedures

The evaluation procedures were applied before the beginning of the study and at the end of the 16 weeks for each patient.

Physical characteristics: Weight and height of each patient were measured with weight and height scale and BMI was calculated using the formula: BMI= weight (kg)/ height (m^2) [11].

The 10-meter walk test (10MWT): To particularly assess and detect the disorder in functional mobility and gait, determine prognosis and assess the difference caused by modality. It is frequently utilized for neurologically affected patients and elderly [11]. Stop watch was used to calculate time during 10 meter walk test.

Diet History Questionnaires III Food Frequency Questionnaire: To assess dietary B12, customized (DHQ III FFQ) which were edited specifically to investigate only potential sources of vitamin B-12 in foods one year ago at the base line for both groups and at the end of 16 week for both groups (A and B). Patients during the first visit learned how to keep detailed weighed food. Food models were used to show examples of how to estimate amounts of food i.e. number of units eaten or portion sizes (e.g., slices cups, pieces and spoons). Interviewer entered responses into the system for patients due to low literacy with English language. The DHQ III analysis program is fully integrated in DHQ web-based software, nutrients and food groups intake based on questionnaire responses are automatically calculated and available on researcher website.

Homocysteine and Vitamin B12 biomarkers:

Patients finished an eight-hour fasting, blood was drawn to assess values of serum vitamin B12 and homocysteine.

Serum Hcy and vitamin B12 levels were measured; Samples were gathered from every patient after overnight fasting. A venipuncture cite was chosen in the antecubital area and a tourniquet was positioned superior to the cubitus to restrain venous outflow. The samples were taken into 5 mL plain evacuated tubes and then were centrifuged, after 30 min of sampling, at 2000 g for 10 min. The serum was collected and stored at 80°C before laboratory analyses [5].

Serum vitamin B-12 was assessed chemiluminescent immunoassay (IMMULITE 2000; Siemens Healthineers Global). A cutoff value of <150 pmol/L was utilized for cobalamin deficiency [12].

Serum homocysteine values were measured with ELISA Kit; a competitive enzyme immunoassay developed for the detection and quantitation of Hcy in plasma.

Mean corpuscular volume (MCV) will be directly measured by automated hematology analyzer [13].

Treatment procedures:

Group (A): consisted of thirty patients received a well-established moderate intensity endurance exercise program. They were asked to warm up and cool down for at least five to seven minutes each, before and after exercise) in the form low intensity of pace walking (walking at low speed 1.5 k.m./h. at 0 inclination). The patients walked on electronic treadmill (Kettler Marathon HS HKS-Selection treadmill) for 30 min (total session) [14], with moderate intensity; 3 MET walking speed [15] which using measured RMR was substantially lower at 2.5 k.m./h. at 0 inclination [16]. Three times / week (day after day) for 16 weeks.

Training patients ate 2600-kcal level DASH diet for 16-week which from chemical analyses of composited meals had 6.5 mg of vitamin B12 [17] with a certain number of daily servings from various food groups. These were given in DASH eating plan chart.

Group (B): consisted of thirty patients treated with IM injection of cyanocobalamin, generally (1 ampoule/day; every ampule contained 1 mg of cyanocobalamin for two weeks, followed by 1 mg/week for 14 weeks [18] under regular coordination and direction by a physician), in order to assure effective treatment.

Statistical analysis:
Data analysis was conducted using SPSS software, the

between the two groups. P-values < 0.05 were considered statistically significant.

3 Results and Discussion

I- General characteristics of the patients:
There was no significant difference (p>0.05) in the mean value of age, weight and height between both groups A and B before treatment (Table 1).

Table 1: Physical characteristics of the patients.

	Group(A) (n=30)	Group (B) (n=11)	t-test [a]	P value**
Age (years)	**68.23±2.74**	**67.83±2.19**	**0.624**	**0.535^{NS}**
Weight (Kg)	**82.42±9.09**	**82.22±9.71**	**0.085**	**0.933^{NS}**
Height (m)	**1.66±0.07**	**1.65±0.07**	**0.423**	**0.674 ^{NS}**
BMI (kg/m²)	**29.86±2.28**	**30.05±2.28**	**-0.321**	**0.749^{NS}**

*Significant level is set at alpha level <0.05 P- value: probability value

[a] : Paired Sample t-test t: Student t-test.

NS p>0.05=no significant p<0.05=Significant

Within group comparison:
Statistical improvements were found in B12 biomarkers and gait speed in both groups A & B by reporting a significant increase in Serum B12, 10MWT and a significant reduction in MCV, tHcy in comparison to the "pre" training (P-value <0.001 for all). There was a significant increase in dietary B12 in study group (P-value =001*) with a non-significant change in control group (P-value =0.1033) in comparison to the "pre" training (Table 2).

Between group comparisons: There was no significant

version 25 (IBM Corp., Armonk., NY., USA).

Shapiro–Wilk test was used to determine whether the data were normally distributed (p>0.05). Continuous data were described as mean± standard deviation (SD). Baseline characteristics between the two groups were compared using the Independent Samples t-test. Paired t-test was conducted to examine the changes in variables before and after the intervention in each group; while an independent samples t-test was conducted to examine the differences

difference between two groups A&B in dietary B12, serum

B12, tHcy, MCV and 10MWT before training (Table 2).
There was significant increase in the mean values of serum B12 between two groups and significant reduction in the serum B12 between two groups and significant reduction in the mean values of tHcy and MCV between two groups after training and these significant differences was in favour of group B (control group) (Table 2).
There was significant increase in the mean values of dietary B12 and 10MWT between two groups after training and these significant differences was in favour of group A (study group) (Table 2).

Table 2: Mean ±SD and p values of Dietary B12, Serum B12, MCV, tHcy and 10MWT pre and post training between and within group comparison.

		Group (A) (n=30)	Group (B) (n=30)	P value***
Dietary B12	Pre-training	**1.59±0.44**	**1.80±0.39**	**0.068[NS]**
(mg/d)	Post-training	**6.57±1.33**	**1.84±0.35**	**<0.001*[S]**
	Improvement **%**	**312%**	**2%**	**<0.001*[S]**
	P value**	**0. 001*[S]**	**0.1033 [NS]**	
Serum B12	Pre-training	**105.36±8.15**	**103.35±9.22**	**0.375[NS]**
(pmol/L)	Post-training	**155.60±13.14**	**242.63±16.92**	**<0.001*[S]**
	Improvement **%**	**%47.68%↑**	**%134.765%↑**	**<0.001*[S]**
	P value**	**<0.001*[S]**	**<0.001*[S]**	
MCV	Pre-training	**111.20±7.46**	**108.13±6.23**	**0.089 [NS]**
(fL)	Post-training	**93.83±3.07**	**88.70±2.22**	**<0.001*[S]**
	Improvement **%**	**%15.62% ↓**	**%17.97%↓**	**0.122 [NS]**
	P value**	**<0.001*[S]**	**<0.001*[S]**	
tHcy	Pre-training	**22.54±2.43**	**23.45±4.06**	**0.295 [NS]**
(μmol/L)	Post-training	**16.02±1.73**	**14.70±1.50**	**0.002*[S]**
	Improvement **%**	**%28.93%↓**	**%37.31%↓**	**0.001*[S]**
	P value**	**<0.001*[S]**	**<0.001*[S]**	
10MWT	Pre-training	**0.93±0.03**	**0.96±0.09**	**0.208[NS]**
(m/s)	Post-training	**1.31±0.06**	**1.04±0.06**	**<0.001*[S]**
	Improvement **%**	**%40.86% ↑**	**%8.33% ↑**	**<0.001*[S]**
	P value**	**<0.001*[S]**	**<0.001*[S]**	

Dietary B12: Dietary Vitamin B12 (mg/d). **Serum B12:** Serum Vitamin B12 (pmol/L). **MCV:** Mean Corpuscle Volume (fL). **tHcy:** Total Homocysteine (μmol/L). **TMWT:** 10-Meter Walk Time (m/s).

*Significant level is set at alpha level <0.05 P- value: probability value

** Within group comparison *** Between group comparison

NS p>0.05=no significant Sp<0.05=Significant

So, it could be concluded that endurance training program in combination with DASH diet were effective in improving homocysteine and vitamin B12 biomarkers of anemia but not as conventional therapy and significantly improved gait speed.

Overall, in this study vitamin B-12 concentrations were deficient (i.e., ≤ 150 pmol/L) with hyper-homocysteinemia (HHcy) and macrocytosis at baseline, in both groups, dietary B12 was deficient at the baseline which may be attributed to the fact that majority of the participants were deficient due to dietary causes, 10MWT was low (i.e., ≤ 1.0 m/s) at baseline, in both groups, which predicts frailty [19].

1. Effect on serum vitamin B12: -

The improvement in Vitamin B12 concentration from baseline is attributed to change of dietary pattern to DASH diet with consequent improvement in quantity of dietary B12 from 0.9 to 6.5 μg daily. This is in agreement with meta-analysis of 29 studies done with adults and 27 studies done with elderly individuals (mean age of elderly >65 y) which showed a valuation of the quantity-response association between cobalamin intake and biological markers of B-12. Based on 56 valuations in 15,968 individuals, the results concluded that increasing cobalamin to the double, elevates plasma values by 11% and a little more in geriatrics [20].

Another explanation is related to gut microbiota ability to produce B12 as only few intestinal microbiotas that can produce cobalamin in the human GIT [21]. About 20% of intestinal microbiota can synthesize cobalamin [22]. The intestinal microbiota vitamin B generation is influenced by a lot of things, including diets having prebiotics [23]. Quantity of fruit and veggies in DASH gives us high amounts of potassium, magnesium and fiber [24] which act as a nutrition for good bacteria and assist their development to produce vitamin B12.

The current study results agreed with Kim et al., [25] who showed that consistent moderate exercise training reduced serum folic acid and elevated serum cobalamin values in moderate exercise trained rats.

The improvement in B12 concentration may also be explained by (Choi and Cho, 2014) [26] who reported that elevation in serum cobalamin values in aerobic exercised rats may be caused by high lipids oxidation as an exercise accommodation as aerobic exercised rats had notably more serum free-fatty acid unlike non exercising ones. Cobalamin action is not similar to that of folic acid, which is included in lipid oxidation to work as a cofactor of Methylmalonyl-CoA mutase.

The results agreed with Groenendijk et al., [12] who examined the influences of a nutrition with training involvement on plasma cobalamin levels in Chinese middle-aged and elderly. A milk supplemental fortification with training involvement elevated serum B12 very well which improved cobalamin 1st values (345 ± 119 pmol/L) to 24 weeks (484 ± 136 pmol/L). The dietary ingested cobalamin was in agreement with the plasma cobalamin values.

Increased dietary intake following physical activity engagement might facilitate body store of B vitamins, so decreasing homocysteine values [5]. Also, systemic review conducted by Mitchell et al., [27] showed that training seems to be linked to diversity in intestinal beneficial bacteria constitution, an elevation in butyrate making bacteria, some of these can make cobalamin [28].

2. Effect on serum homocysteine: -

The results agreed with Appel et al., [17] who found that DASH combination diet for 8- weeks trial had a positive influence on fasting values of total homocysteine as it supplied more cobalamin than either the control diet or the fruits and veggies diet.

The results agreed with meta-analysis of variant 34 trials clarified that frequent PA is related to reduction in Hcy values and that training programs could effectively influence Hcy regulation. [29].

The results agreed with Deepak and Dwivedi, [30] who chose 100 participants and 223 control, measured Hcy values and PA. They found that training 20 mins per day decreases HTN in about 5 days, it was also so good to decreases Hcy values remarkably in physically active individuals 12.19 ± 2.72 μmol/L as related to sedentary 17.27 ± 2.12 μmol/L particularly elderly diagnosed with hypertension and all hemiplegic patients.

On the other Alomari et al., [5] who showed the value of the vitamins for controlling homocysteine values. Their results showed that plasma homocysteine value was decreased in elderly men and women, and young men constantly engaging in physical activity without influencing and unassociated with B vitamins which disagree with our results.

The results regarding influence of exercise on reducing hyper-homocysteinemia is attributed to facilitation of antioxidant capacity, decreases Matrix metallopeptidase 9 and stops degradation of Extracellular matrix, reduces M1/M2 macrophage ratio and inflammation, and facilitates white fat cells, which are important for decreasing homocysteine [31]. Also, E Silva and Da Mota, [29] found that for aerobic exercise programs, reduced PH is anticipated, since aerobic exercise programs elevates protein turnover and antioxidant capacity. Actually, aerobic training facilitates hepatic glutathione generation, which supports the reduction in Hcy.

The significant reduction in tHcy observed in group A, was not enough to reach optimal biomarker level. We postulated, that; it was needed to increase the trial period as more decline might be tracked.

Group B showed more improvement in serum B12 and Hcy values which may be expected because in study group A, cobalamin absorption is minimal as in a single meal intake, the upper cut of absorption relies on the saturation of transport system, which is able to bind only 1.5- 2μg [32], whereas in control group B, about 10% of the injected amount (100 of 1000 μg) is kept. Hematologic feedback is fast, with an elevation in the immature red blood cells value in 1 wk and treatment of megaloblastic anemia in 6 to 8 weeks [33].

3. Effect on gait speed: -

The current results agreed with Roma et al., [34] who showed that non exercising older individuals who did walking activity for 30 minutes 2 times a week for 12 months had a statistical improvement in walking speed, Henderson et al., [35] who found that both aerobic exercises and weight training exercises increased habitual-pace walk speed, only aerobic exercises improved quick-pace walk speed for overweight and fat non exercising older individuals.

In preceding trials, walking speed in its own has widely considered a simple indicator of the physical abilities and evaluator of the frailty of the older individual population [19].

Suggested criteria for clinically meaningful alteration when evaluating the habitual walking speed of community dwelling elderly evaluated through 4 to 10 m is 0.05 m/s for minor meaningful alteration and 0.1 m/s for considerable meaningful alteration [36].

The mean difference of post-pre values of gait speed in control group was 0.09 m/s which is a small meaningful change compared with study group mean difference of post-pre value 0.38 m/s which is a substantial meaningful change and reduce risk of frailty in favor to study group indicating the importance of endurance training in treating neurological aspect of anemia.

Although group B showed more improvement in serum B12 & Hcy results than study group but study group showed more significant improvement in 10MWT this may be explained by Soh et al., [19] who conducted a cross-sectional study, to examine the association between frailty and cobalamin values in community-dwelling Korean elderly.

They revealed that B12 insufficiency elevated the occurrence of frailty and influenced physical functions, but it doesn't elevate the occurrence of frailty when factors affecting both frailty and B12 insufficiency are taken into account. Frailty is induced by a lot of factors not just 1 thing, and cobalamin is just a part of these factors. Also neurologic manifestations of megaloblastic anemia resolve over weeks to months as a result of injected cobalamin [33].

4 Conclusions

It can be concluded that endurance training program in combination with DASH diet were effective in improving homocysteine and vitamin B12 biomarkers of anemia but not as conventional therapy and significantly improved gait speed

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