جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب البيطري

***Association between vitamin B6 vitamin B12 folic acid intake and Effects of Excess Vitamin Intake on Serum Biochemical Markers in***

***rats***

بحث مقدم الى كلية الطب البيطري كجزء من متطلبات نيل شهادة البكالوريوس

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بسم الله الرحمن الرحيم

(( اقرأ باسم ربك الذي خلق ۞ خلق الانسان من علق ۞ اقرأ وربك الاكرم ۞ الذي علم بالقلم ۞ علم الانسان ما لم يعلم ۞ ))

صدق الله العظيم

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الاهداء

الى أمي وأبي

الى أهلي وعشيرتي

ألى أساتذتي

الى زملائي وزميلاتي

الى الشموع التي تحترق لتضئ للأخرين

الى كل من علمني حرفا

أهدي هذا البحث المتواضع راجيا من المولى

عز وجل أن يجد القبول والنجاح

**Abstract**

The present study was undertaken to investigate the effects of dietary excess of vitamin B6, B12, Folic acid on certain blood parameters [serum totalprotein, Creatinine,Albumin] and the A total of 48 rats were included in the study. Saline solution was administered to control groups (CG-10, n = 8 for 10 days; CG-15, n = 8 for 15 days; CG-20, n = 8 for 20 days). The experimental groups (EG-10, n = 8; EG-15, n = 8; EG-20, n = 8) received 5 mg/kg vitamin B6 daily for 10 days, 15 days and 20 days, respectively. Serum Parameters levels were measured and compared in CGs and EGs.

in rats who received long–term high doses of vitamin B6 B12, Folic acid Based on these results, a relationship between levels in the EG-15 and EG-20 groups is suggested Conclusions: Dietary excess of vitamin B6 B12,Folic acid intake reduces serum total Protein and another parameters levels,. Thus, a careful diet plan and monitoring of vitamins dose are recommended in patients who are supplemented with this vitamins.

**INTRODUCTION**

**INTRODUCTION:**

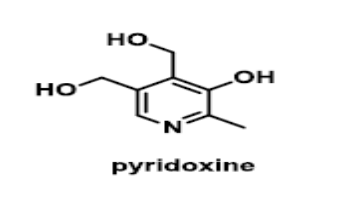
Nutritional deficiencies have been documented to affect cellular differentiation during development. Several cognitive dysfunctions have been reported in subjects with nutritional deficiencies in basic elements, including pellagra syndrome, depression, irritability, confusion and disorientation resulting from niacin deficiency, and Wernicke-Korsakoff syndrome resulting from thiamine deficiency. Vitamin B6 has been shown to be important for normal cognitive function and in lowering the incidence of coronary heart disease among the elderly(1,2,3)

***vitamin B6***

The effects of malnutrition on organogenesis are well documented (4&5), even though discussion on the basic mechanisms still continues (6&10). The morphological changes observed in developing brain regions associated with maternal vitamin B6 deficits were reported in rodents (4&11). Gutierrez-Reyes et al. (12) have indicated that malnutrition of vitamin B6 also causes changes in memory efficiency in rats. Dysfunctions in the immune system are related to malnutrition status (13). Structural cell changes in different organs associated with maternal vitamin B6 deficiency have been reported during development. It has been suggested that vitamin B6 restriction during gestation periods was a potential risk factor for a defect

in synaptogenesis and neural differentiation (14&15)). During pregnancy and lactation, this deficiency altered the function of N-methyl-D-aspartate receptors, important glutamatergic compounds involved in learning and memory (16).

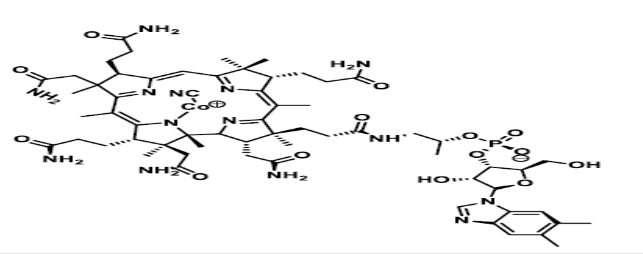
**Chemical Stricture VitB6**

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***Vitamin B12***

Vitamin B12 is the generic name for a specific group of cobalt-containing corrinoids with biological activity in humans. This group of biologically active corrinoids is also described as cobalamins. Cyanocobalamin is the commercialy available form used in food supplements and food fortification. In foods, hydroxo-, methyl- and 5'-deoxyadenosyl-cobalamins are the main cobalamins present. Sulphitocobalamin, with a sulphite ligand chelated to the central cobalt atom in the corrin ring, may occur is some processed foods. Vitamin B12 functions primarily as a coenzyme in intermediary metabolism. Only two vitamin B12 dependent reactions have been identified thus far for humans: 1) the methionine synthase reaction with methylcobalamin, and 2) the methylmalonyl CoA mutase reaction with 5- deoxyadenosylcobalamin as the active coenzyme, respectively. A dietary vitamin B12 deficiency can occur in strict vegetarians or after gastrectomy, and other diseases affecting cobalamin absorption. About two-thirds of patients with vitamin B12 deficiency have pernicious anemia (PA), an autoimmune disorder associated with gastric atrophy and absence of Intrinsic Factor (IF) which results in vitamin B12 malabsorption. The key symptom in vitamin B12 deficiency is macrocytic megaloblastic anemia.These haematological abnormalities are indistinguishable from those seen in folate deficiency, because of the interrelated function of both vitamins (18). Another key symptom of vitamin B12 deficiency are neurological complications, such as paraesthesia, leg weakness, memory loss, etc, due to progressive lesions in the lateral and posterior columns of the spinal cord subacute combined degeneration of the spinal cord). Neurological symptoms occur in about75-%90 of all individuals with (untreated) vitamin B12 deficiency, and appear generally at a later stage. In about 25% of all cases neurological symptoms are the only symptoms, i.e. without haematological abnormalities(19).

**Chemical Stricture B12**

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***Folate and folicacid***

Role of folate and folic acid in human metabolic processes olates accept one-carbon units from donor molecules and passes them on via various biosynthetic reactions (20). In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one-carbon forms of tetrahydrofolate (e.g., 10- formyl, 5,10-methylene, and 5-methyl) (Figure 2). The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and p-aminobenzoylglutamate, which have no biologic activity (21). Substituting a carbon group at N-5 or N-10 decreases the tendency of the molecule to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity). The folates found in food consist of a mixture of reduced folate polyglutamates.(22)

**Figure 2**

**The chemical formula of folic acid (synthetic form)**

**and the most important natural folates. (17)**

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The purpose of this study was to investigate whether high doses of vitamins B6, B12, Folic acid affect the levels of serum total protein , Albumin and Creatinine level, and especially to compare the levels of control and experimental groups in a time-dependent manner. (23)

**Materials and Methods**

**Materials and Methods**

* **Experiments:**

The study protocol was approved by the Baghdad University Animal Care Center. Since there is a difference in nerve conduction velocity between males and females(38), we used only healthy male albino rats obtained from the animal care center of the Medical Faculty of Akdeniz University. Animals were housed in groups of four rats in stainless steel cages under standard conditions (24 ± 2°C and 50 ± 5% humidity) with a 12 h light-dark cycle (37). Six rats were used for each of three control groups (CG-10, CG-15, CG-20; total = 24) and three experimental groups (EG-10, EG-15, EG-20; total = 24). Thus, totally48 Swiss Albino rats weighing 200-250 g were used for the study. The National Research Council (NRC) recommends a 7 mg/kg dose for vitamin B6, dissolved in

physiologic saline solution (0.9% NaCl), but previous studies administered higher than the recommended doses). The dose chosen in this study (5 mg/kg) is similar to that applied in previous studies (24&25)

Vitamin B6 (5 mg/kg/day) was injected intraperitoneally IP) to the experimental groups EG-10, EG-15 and EG-20 for 10, 15 and 20 days, respectively. Physiologic saline solution was injected for 10, 15 and 20 days to the control groups, CG-10, CG-15, CG-20, respectively. Treatments for all groups, including daily doses and time periods, are summarized in Table 1.(26)

Table 1. Doses of daily administered vitamin B6 and saline according to

experimental groups (EGs) and control groups (CGs)).

----------------------------------------------------------------------------- Groups Experiment period (days) Dailydose

EG-10(n-8) 10 vitamin B-6 5mg\kg

CG-10(n-8) 10 0.9%NaCl 5mg\kg

EG-15(n-8) 15 vitamin B-6 5mg\kg

CG-15(n-8) 15 NaCl 0.9% 5mg\kg

EG-20(n-8) 20 vitamin B-6 5mg\kg

CG-20(n-8) 20 NaCl 0.9% 5mg\kg

**Blood sample analysis**

The blood samples were collected from each group viacardiac puncture. The levels of total Protein, Albumine and Creatinine index in serum were determined in a Dacos Autoanalyzer using enzymatic Dort reagents (Counter Inc Hielab, Miami, USA). All parameters level in different EGs were compared with each other and CGs(27).

**Parameters Procedures**

***1-Creatinine***

**Procedure**

1-Preincble working reagent, sample and standard to reaction temperature (37˚C).

2-Set the photometer to 0 absorbance with distilled water.

3-Pipette into a cuvette.

|  |  |
| --- | --- |
| 1.0mL | Working reagent |
| 10 µL | Sample or standard |

4-Mix gently. Insert cuvette into the temperature controlled instrument and start stopwatch.

5-Record absorbance at 510 nm after 30 second (A1) and after 90 second (A2) of the sample or standard addition.

CALCULATION

(A2-A1)Sample × C standard =mg/dL creatinine

(A2-A1)Standard

Mg/dL × 88.4=µmol/L

(28)

**2-Albumin**

*Procedure:*

Assay condition:

WavelengthP: ………………………………630 (600-650) nm.

Cuvette: …………………………………………1 cm light path.

Temperature: ……………………………………. 15-25˚C.

Adjust the instrument to zero with distilled water.

Pipette into a cuvette:

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Standard | Blank |  |
| 1.0 | 1.0 | 1.0 | R(mL) |
| -- | 5 | -- | Standard(µL) |
| 5 | -- | -- | Sample(µL) |

Mix and incubate 10 min at 37˚C or 10 min at room temperature(15-25˚C).

Read the absorbance (A) of the sample and standard, against the Blank. The colour is stable 1 hour at room temperature.

CALCULATIONS

(A)Sample ×7 (Standard conc.) =g/dL of albumin in sample

(A)Standard

Conversion factore: g/dL × 144.9 =µmol/L

(29)

**3-Total protein**

*Procedure:*

Assay condition:

WavelengthP: ………………………………540 (530-550) nm.

Cuvette: …………………………………………1 cm light path.

Temperature: …………………………………….37˚C / 15-25˚.

Adjust the instrument to zero with distilled water.

Pipette into a cuvette:

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Standard | Blank |  |
| 1.0 | 1.0 | 1.0 | R(mL) |
| -- | 25 | -- | Standard(µL) |
| 25 | -- | -- | Sample(µL) |

Mix and incubate 5 min at 37˚C or 10 min at room temperature.

Read the absorbance (A) of the sample and standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

(A)Sample ×7 (Standard conc.) =g/dL of total protein in sample

(A)Standard

(30)

***Results***

***&***

***Discussion***

***Results***

**Blood parameters:**

Total protein . Albumen and Creatinine levels were determined for all CGs. No significant differences were observed among the CGs for these values. Thus, the

mean values for each parameter of the three control groups were accepted as the normal values and compared with the mean values of each parameter in EGs (Table 2). The blood levels of Biochemical parameters according to EGs and CGs are presented comparatively in Table 2.

**Table 2. Blood parameters from experimental groups (EGs) treated with high- dosevitamin B6. The effects of vitamins administration on serum Total Protein Albumin and Creatinine levels are comparable between EGs and control groups** (CGs).

Total Protein Albumin Creatinine

(mg/dl) (mg/dl) (mg/dl)

CGs mean value 6.6 4.34 38.5

CG-10 (n = 8) 7.5 4.2 28.5

CG-15 (n = 8) 7.0 4.0 22.7 CG-20 (n = 8 ) 7.1 4.02 25

EG-10 (n = 8) 4.0 6.8 100

EG-15 (n = 8) 3.5 7.7 110

EG-20 (n = 8) 3.5 8.0 120

**Discussion**

**Discussion:**

For the past two decades, there have been suggestions that abnormalities in cellular function might be related to vitamins deficiency. Vitamins is closely related with the permeability of the cellular membranes and a secondary interaction takes place between the intracellular Krebs cycle and cholesterol levels. For instance, in vitamins deficiency, (31&32). The administration of high doses of vitamin B6 is effective in the suppression of hepatic cathepsin B activity(33). In a study with dialysis patients who were supplemented with vitamin B6, a reduction in

homocysteine levels and improvement in the lipidemic profile of the patients were reported (34). Dietary excess of vitamin B6 supplementation supports the hypothesis that aldehydes are involved in increased The results of our recent study showed that long-term administration of high doses of vitamin B6 caused cellular and neuropilar damage and loss of some cellular organelles in the cerebral cortex. In addition, the pyramidal neurons suffered proportional atrophy of cellular processes and components.(35)

In this study we saw decrease Total Protein level in all cases but Increase in Control group. Creatinine level and Albumin increase in all patients groups but decrease in control groups.

**REFERENCES**

**REFERANCES:**

1. Bryan J, Calvaresi E, Hughes D. Short-term folate, vitamin B-12 or vitamin B6 supplementation slightly affects memory performance but not mood in women of various ages. J Nutr2002; 132: 1345-1356.

2. Calvaresi E, Bryan J. B vitamins, cognition and aging: a review. J Gerontol B Psychol Sci Soc Sci 2001; 56: 327-339.

3. Fletcher RH, Fairfield KM. Vitamins for chronic disease prevention in adults: clinical applications. JAMA 2002; 287: 3127-3129.

4. Morre DM, Kirksey A. The effect of a deficiency of vitamin B6 on

selected neurons of the developing rat brain. Nutr Repro Int

1980; 21: 301-312.

5. Scheibel ME, Scheibel AB. Structural alterations in the aging brain.

In: Danon D, Shock NW, Marios M, editors. Aging. Oxford: Oxford

University Press; 1991. pp. 4-17.

6. Dakshinamurti K, Sharma SK, Geiger JD. Neuroprotective actions

of pyridoxine. Biochim Biophys Acta 2003; 1647: 225-229.

7. Dolina S, Peeling J, Sutherland G, Pillay N, Greenberg A. Effect of

sustained pyridoxine treatment on seizure susceptibility and

regional brain amino acid levels in genetically epilepsy-prone

BALB/c mice. Epilepsia 1993; 34: 33-42.

8. Ebadi M, Jobe PC, Laird HE 2nd. The status of vitamin B6

metabolism in brains of genetically epilepsy-prone rats. Epilepsia

1985; 26: 353-359.

9. Schaeffer MC, Gretz D, Mahuren JD, Coburn SP. Tissue B-6

vitamin concentrations in rats fed excess vitamin B-6. J Nutr

1995; 125: 2370-2378.

10. Sharma SK, Dakshinamurti K. Determination of vitamin B6 vitamers and pyridoxic acid in biological samples. J Chromatogr1992; 578: 45-51.

11. Sharma SK, Bolster B, Dakshinamurti K. Picrotoxin and pentylene tetrazole induced seizure activity in pyridoxine-deficient rats. J Neurol Sci 1994; 121: 1-9.

12. Gutierrez-Reyes E, Castaneda-Perozo D, Papale-Centofanti J. Supersensitivity of the cholinergic muscarinic system in the rat's brain is induced by high concentrations of Cu+2. Invest Clin2002; 43: 107-117.

13. Calder PC, Kew S. The immune system: a target for functional foods? Br J Nutr 2002; 88: 165-177.

14. Groziak SM, Kirksey A. Effects of maternal dietary restriction in vitamin B-6 on neocortex development in rats: B-6 vitamin concentrations, volume and cell estimates. J Nutr 1987; 117: 1045-1052.

15-Herbert V (1987). The 1986 Herman Award lecture. Nutrition science as a continually unfolding story: the folate and vitamin B-12 paradigma. Am J Clin Nutr 46: 387-402.

16-Bower C and Wald NJ (1995). Review: Vitamin B12 deficiency and the fortification of food with folic acid, E J Clin Nutr 49: 787-793.

17-FAO/WHO expert consultation on human vitamin and mineral requirements Page93 Human Vitamin and Mineral Requirements by Report of a joint FAO/WHO expert consultation Bangkok, Thailand - FAO 2001

18. Bryan J, Calvaresi E, Hughes D. Short-term folate, vitamin B-12

or vitamin B6 supplementation slightly affects memory

performance but not mood in women of various ages. J Nutr

2002; 132: 1345-1356.

19. Calvaresi E, Bryan J. B vitamins, cognition and aging: a review. J

Gerontol B Psychol Sci Soc Sci 2001; 56: 327-339.

20. Morre DM, Kirksey A. The effect of a deficiency of vitamin B6 on

selected neurons of the developing rat brain. Nutr Repro Int

1980; 21: 301-312.

21. Scheibel ME, Scheibel AB. Structural alterations in the aging brain.

In: Danon D, Shock NW, Marios M, editors. Aging. Oxford: Oxford

University Press; 1991. pp. 4-17.

22. Dakshinamurti K, Sharma SK, Geiger JD. Neuroprotective actions

of pyridoxine. Biochim Biophys Acta 2003; 1647: 225-229.

23. Dolina S, Peeling J, Sutherland G, Pillay N, Greenberg A. Effect of

sustained pyridoxine treatment on seizure susceptibility and

regional brain amino acid levels in genetically epilepsy-prone

BALB/c mice. Epilepsia 1993; 34: 33-42.

24. Ebadi M, Jobe PC, Laird HE 2nd. The status of vitamin B6

metabolism in brains of genetically epilepsy-prone rats. Epilepsia

1985; 26: 353-359.

25. Schaeffer MC, Gretz D, Mahuren JD, Coburn SP. Tissue B-6

vitamin concentrations in rats fed excess vitamin B-6. J Nutr

1995; 125: 2370-2378.

26. Sharma SK, Dakshinamurti K. Determination of vitamin B6

vitamers and pyridoxic acid in biological samples. J Chromatogr

1992; 578: 45-51.

27. Sharma SK, Bolster B, Dakshinamurti K. Picrotoxin and pentylene

tetrazole induced seizure activity in pyridoxine-deficient rats. J

Neurol Sci 1994; 121: 1-9.

28. Gutierrez-Reyes E, Castaneda-Perozo D, Papale-Centofanti J.

Supersensitivity of the cholinergic muscarinic system in the rat's

brain is induced by high concentrations of Cu+2. Invest Clin

2002; 43: 107-117.

29. Calder PC, Kew S. The immune system: a target for functional

foods? Br J Nutr 2002; 88: 165-177.

30. Groziak SM, Kirksey A. Effects of maternal dietary restriction in

vitamin B-6 on neocortex development in rats: B-6 vitamin

concentrations, volume and cell estimates. J Nutr 1987; 117:

1045-1052.

31. Groziak SM, Kirksey A. Effects of maternal restriction in vitamin

B-6 on neocortex development in rats: neuron differentiation and

synaptogenesis. J Nutr 1990; 120: 485-492.

32. Guilarte TR. Vitamin B6 and cognitive development: recent

research findings from human and animal studies. Nutr Rev

1993; 51: 193-198.

33. Abbas ZG, Swai AB. Evaluation of the efficacy of thiamine and

pyridoxine in the treatment of symptomatic diabetic peripheral

neuropathy. East Afr Med J 1997; 74: 803–807.

34. Lee NS, Muhs G, Wagner GC, Reynolds RD, Fisher H. Dietary

pyridoxine interaction with tryptophan or histidine on brain

serotonin and histamine metabolism. Pharmacol Biochem Behav

1988; 29: 559-564.

35. Ravichandran V, Selvam R. Increased lipid peroxidation in kidney

of vitamin B6-deficient rats. Biochem Int 1990; 21: 599–605.

**الخلاصة:**

فيتامين B12، B6 والفولك فيتامينات ذائبة في الماء تفرز الزائدة منها في البول , ان زيادة تناول الفيتامينات وجد لها تاثيرات فسلجية على وضائف الجسم،

الهدف: أجريت هذه الدراسة للتحقيق في تأثير الزائد الغذائي من فيتامين B6 ، B12 الفوليك اسد في الدم كمؤشرات للبروتين في الدم، والدهون النافعة ، الكوليسترول والدهون الكلي الالبومين والكرياتينين.

تم العمل على مجموع 48 من الجرذان في الدراسة. تم إعطاء محلول ملحي للمجموعات السيطرة (CG-10، n = 8 لمدة 10 أيام؛ CG-15، n = 8 لمدة 15 يوما؛ CG-20، n = 8 لمدة 20 يوما). وقد تلقت المجموعات التجريبية (EG-10، n = 8؛ EG-15، n = 8؛ EG-20، n = 8) 5 ملغم / كغم فيتامين B 6، B 12 يوميا لمدة 10 أيام و 15 يوما و 20 يوما على التوالي. تم قياس ومقارنة البروتين الكلي في الدم ، و HDLالالبومين والكرياتينين في مجموعة السيطرة والتجريبية. النتائج: كانت مستويات البروتين الكلي في الدم أقل بشكل ملحوظ في مجاميع السيطرة.عن المجاميع التجريبية وكانت مستويات الالبومين والكرياتينين في مصل الدم أعلى في المجموعتين EG-15 و CG-20 مقارنة بالمجموعات اخرى. وبناء على هذه النتائج، ان ومستويات المتغيرات الكيموحيوية في المجموعتين CG-15 و CG-20. تاثرت بشكل ملحوظ بعد الزيادة بجرع الفيتامينات. الاستنتاجات: الزائد من تناول فيتامين B6، B12 يؤثر في مستويات البروتين الكلي في الدم، ، وبالتالي، ينصح في خطة النظام غذائي ان يؤخذ بجرع دقيقة وضبط جرعة فيتامين B6 في المرضى الذين يحتاجون هذا الفيتامينات.