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New Vitamin B12 Buccal Films in Patients with Liver Cirrhosis Adjuvant with Diabetes Mellitus

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Abstract

Objective: To determine prospectively plasma levels of vitamin B12 in diabetic patients with liver cirrhosis before and 3 months after vitamin administration and its effects on nutritional state of patients. Additionally study a new dosage form of vitamin B12 (buccal film) in compare to traditional one as intramuscular injection.

Methods: 120 blood samples were collected from 60 adult cirrhotic patients for estimation of vitamin B12 levels before and after vitamin administration. Additionally there was an estimation of other biochemical parameters to study the effect of B12 level changes on them. The most changeable parameters were hemoglobin, AST, ALT, and Transferrin levels. The rate of change in vitamin B12 levels and other biochemical parameters was significant before and after treatment with vitamin, adds to the difference in levels between the two different dosage forms groups. Vitamin B12 levels were measured by EIISA, before and after 3 months from the completion of treatment.

Results: Vitamin B12 serum concentrations did not show any significant differences among the two groups before treatment. There was a significant increase in vitamin B12 serum concentrations after four weeks of B12 treatment in the two groups (35 ± 26_ 141 ± 49.3 *vs*. 53.8 ± 45.9_ 172.2 ± 52.8 pg/dL,) group I and group II respectively. Paired analysis in each group showed a significant increase for vitamin B12 in the two groups. Statistically significant differences were found for some biochemical parameters as hemoglobin, AST, ALT, and Transferrin concentrations before and four weeks after treatment.

Conclusions: Patients with symptomatic infection by liver cirrhosis and diabetic have a lower vitamin B12 levels than normal. This could reflect a more need for vitamin B12 treatment for those patients for long time. These results could present the opportunity to treat these cases and to use vitamin B12 supplementation. There are many dosage forms for B12 in the markets; here we introduced a new competitor dosage form that was buccal films.

Keywords: Vitamin B12; Liver cirrhosis; Buccal films; Diabetes

Introduction

The exact vitamin requirements of patients with liver cirrhosis with diabetes have not been established. If an adequate, well-balanced diet is taken, it is probable that vitamin requirements are being met. On the other hand, the addition of multivitamin to the diet causes no harm and provides additional assurance that normal and even increased vitamin requirements are being met. A principal advantage of vitamin B12 is that it may be instrumental as an appetite stimulant in certain patients with anorexia, and possibly in this manner be effective in shortening convalescence. As with other vitamins, the side effects of vitamin B12 are minimal. The aim of this study was to investigate vitamin B12 serum concentrations in diabetic cirrhotic patient before and after administration of B12 and the response of cases after treatment. This response included some biochemical factors of malnutrition and some of clinical manifestations.

Materials and Methods

Patients

120 blood samples were collected from 60 Egyptian people (male and female) from Minya (southeast Egypt) during a 12-month period. From the whole population studied, 85 patients were included, 25 of them were excluded from the study: 12 (44.28%) didn't follow the study roles and 5 of them because of no adequate clinical or microbiological control and 8 died. Sixty patients were diagnosed with liver cirrhosis and diabetes mellitus and were finally included in the study. From these 60 cases, we randomly divided into two groups with different vitamin B12 dosage forms. Group I adapted for intramuscular injection where Group II adapted for buccal films prepared by staff members of pharmaceutics, faculty of pharmacy, Deraya University [1]. Most of these patients (volunteers) were of a medium socio-economic status. The majority of them complained of abdominal pain, acute diarrhea, anorexia, anal other clinical manifestations which included in our study. Ages at diagnosis ranged from 18-80, mean 60.4 ± 10.8 years. The study included 60 diabetic cirrhotic individuals (35 males and 25 females) who entered the 4-week vitamin B12 study their demographic data as age, sex and BMI are collected and no statistical

difference between patients in the two study groups in their demographics.

Diagnosis of cases

All patients underwent the following tests: Complete blood cell count, aspartate aminotransferase, alanine aminotransferase, g-glutamyltranspeptidase, alkaline phosphatase, total and direct bilirubin, albumin, prothrombin activity, and some malnutrition parameters (Table 1) adds to clinical manifestations. Ultrasound-guided, fine-needle liver biopsy was performed in all cases. The threshold of adequacy for histological assessment was the presence of more than five portal tracts. Liver cirrhosis was diagnosed by an experienced liver pathologist. Those having serum vitamin B12 level less than 200 pg/ml were enrolled in this study. Serum vitamin B12 level was measured in 60 patients with prior permission from Ethics Committee.

Study design

Patients participating in the study will first undergo a routine check-up as an outpatient. They will be asked to provide blood and urine samples for laboratory testing and will undergo an ultrasound of the liver. Ultrasound examinations use sound waves to determine the size and texture of the liver. After the initial visit subjects will be requested to take Cobalamin (two different dosage form) and follow-up after one week at the outpatient department for a similar check-up.

Additional tests may be requested throughout the study to provide information for other research studies and individual consent will be requested.

Studies examining the role of vitamin B12 in the prevention nutritional deterioration of liver diseases, because the dose of vitamin B12 used in those studies may be different from the dose used to treat vitamin B12 deficiency and the majority of patients included in these studies are not vitamin B12 deficient [2,3].

Measurement of vitamin B12

Vitamin B12 levels were assessed by ELISA [4] at biochemistry department, Deraya university. Values were measured and evaluated twice, first at diagnosis and in a second control one month after the treatment and without active infection.

Statistical Analysis

Sample size calculation and statistical analysis

Sample size of 30 for each group was calculated in the ratio of drug effect in vitamin B12 group and standard group. Due to the skewed distribution of the parameters under study (ALT, AST, vitamin B12), their values were transformed using the natural logarithm. The transformed variables had an approximately normal distribution and the parametric tests assuming a normal distribution were used. On the logarithmic transformed variables, the Pearson's correlation coefficient and the corresponding coefficients of the linear regression were calculated. Independent sample t test for quantitative data between the two groups, Chi Square test for qualitative data between the two groups, Wilcoxon signed rank test and Fisher exact test all were used for qualitative data between the two groups. In all cases, statistical significance was declared for p-values <0.05.

Results

The descriptive measures of the parameters upon admission were significantly differs after B12 treatment in the two groups of study. Vitamin B12 increased significantly in the two groups with 369.5% rate of change. The mean values of the hepatic enzymes in participants in the two groups. Results were: AST between 92.3 ± 51.2 and 95.5 ± 50.6 U/L (normal values: 7-40 U/L), ALT was between 69.7 ± 29 and 70.8 ± 30.1 U/L (normal values: 7-40 U/L). B12 levels were between 35 ± 26 and 63.8 ± 45.9 pg/mL (normal values: 240–1100 pg/mL) on admission.

After four weeks of treatment; Vitamin B12 showed a positive and significant increase and correlation with all measured parameters: AST ($r=0.66$, $p<0.01$), ALT ($r=0.666$, $p<0.01$), B12 ($r=0.4877$, $p<0.001$). In all cases, an increase in each one of the predictors resulted in increasing values of vitamin B12 on admission. However, the predictors were also correlated with each group of patients. Thus, in a multiple linear regression, the only variable that independently predicted the values of vitamin B12 was renal functions as Na^+ and K^+ values.

Results illustrated all parameters; the renal, hematological, liver functions parameters and some clinical manifestations that may affect by cyanocobalamin administrations in diabetic cirrhotic patients. At the beginning there is a descriptive study of patients in the two groups about their renal functions, hematological, liver enzymes and B12 level with some of malnutrition parameters.

This descriptive study shows no significant difference between the two groups in their characters before drug administration in the two different forms in the two groups. Liver enzymes show p=0.8-0.9 (no significant difference) in measurement of ALB, AST, ALT, and bilirubin levels. Blood components as WBCs, RBCs and HB shows p=0.1-0.8 (no significant difference) between patients in the two groups of study.

Post treatment there are some characters changed markedly after vitamin B12 administration than the pre-treatment with the two different methods of administrations. Liver enzymes show smooth decrease in their levels in post-treatment in the two groups as seen in Figure 1. Albumin, transferrin and retinol binding protein also founded to be increased in the two groups post treatment as shown in Figure 2. All clinical manifestations show a great progress with reduction in their severity after treatment with vitamin B12 in both groups as seen in Figures 3-5.

Parameters differs significantly between the two groups of study were vitamin B12, transferrin, MCV and MCH. Group II (buccal patches) achieve positive results upon Group I in vitamin B12 level with p-value 0.024*, transferrin level with p-value 0.025*. Other parameters also like RBCs show significant increase in group II than group I with p-value 0.024*. MCH and Reticulocyte level show significant difference in group II than Group I with p value=0.034* and 0.004* respectively (Table 2).

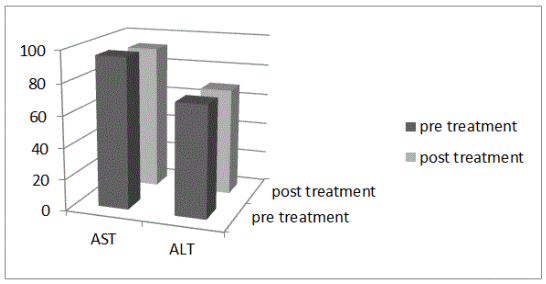

Figure 1: Changes in liver enzymes post-treatment with vitamin B12.

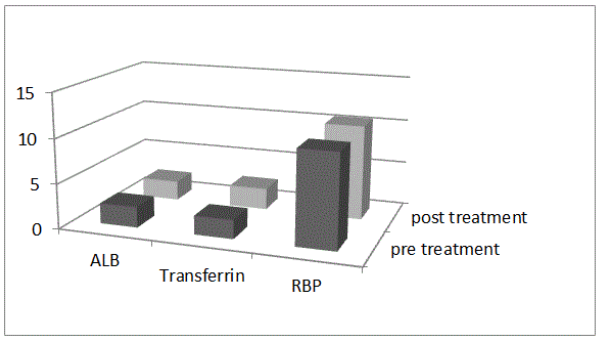

Figure 2: Changes in albumin, transferrin and retinol binding prost-treatment with vitamin B12.

	Pretreatment	Post-treatment	P value
Vitamin B12 Range Mean ± SD	(2.9-81.6) 35 ± 26	(60.8-237.2) 141 ± 49.3	<0.001*
Transferrin Range Mean ± SD	(1.3-2.4) 1.8 ± 0.3	(1.5-2.8) 2.1 ± 0.4	<0.001*
Retinol binding protein Range Mean ± SD	(4.5-17) 9.9 ± 3.2	(4-17.2) 10.7 ± 3.1	0.046*
Lymphocytes Range Mean ± SD	(610-1234) 930.4 ± 150.8	(766-1358) 1082.6 ± 172.5	<0.001*

Table 1: Comparison between pretreatment and post-treatment in the degree of malnutrition (Group I): Paired sample t test, *: Significant difference at p value<0.05.

Clinical manifestations post-treatment results in the two groups of patients

Gastrointestinal problems were easy to be managed and characterized by the practitioners in the department. Sore tongue was one of the most gastrointestinal symptoms that show a great progress post-treatment in the two groups as seen in Figure 3.

Also appetite loss shows a great progress in after vitamin B12 administrations in the two groups of study with no significant differences in results. Weakness is a neurological symptom that can be managed in the two groups of study with no significant differences and show sharp progress. Weakness in Figure 4 show significant increase in group II than Group I with P value equals 0.002*.

Palpitation and Shortness in breath is another symptom that shows a great progress post-treatment with vitamin B12 in the two groups of study. Buccal films show higher compliance than injection as in Figure 5.

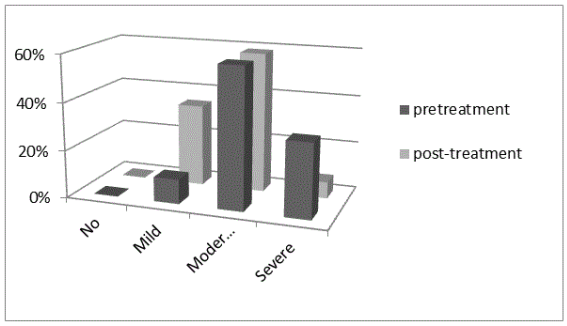

Figure 3: Progress in sore tongue post-treatment with vitamin B12.

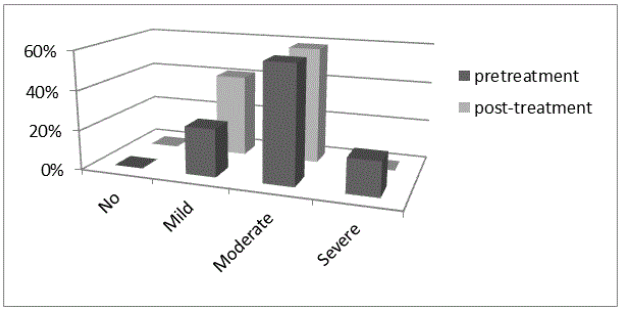

Figure 4: Progress in weakness post-treatment with vitamin B12.

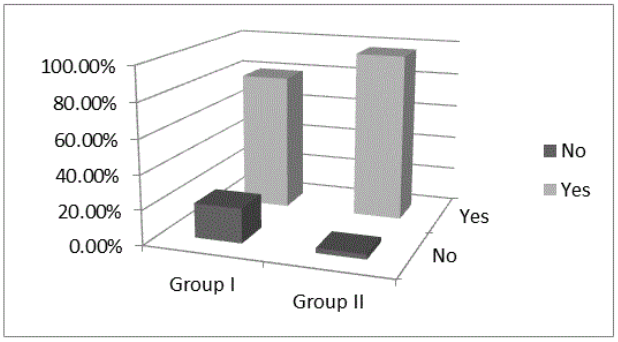

Figure 5: Patients compliance with different methods of administrations in group I and group II.

Parameters improved	Group I			Group II		
	pre-treat	post-treat	Rate of change %	pre-treat	post-treat	Rate of change %
B12 level	35 ± 26	141±49.3	402	53.8±45.9	172.2±52.8	337
MCV	101.5 ± 14.9	88.3 ± 12.7	87.12	95.9±15	89.8±8.9	93
MCH	27.6 ± 4.6	24.4 ± 4.1	88.4	27.4±4.5	26.7±3.6	97.44
Hb	8 ± 1.7	8.9 ± 1.5	111.25	8.2 ± 1.7	9.4 ± 1.4	114.6
Transferrin	1.8 ± 0.3	2.1 ± 0.4	116.6	2.1 ± 0.6	2.4 ± 0.4	114.2
Reticulocytes	0.9 ± 0.8	1.3 ± 0.6	144.44	0.7 ± 0.6	0.8 ± 0.6	114.2
ALT	70.8 ± 30.1	68.6 ± 28.3	96.8	70.8 ± 30.1	68.6 ± 28.3	96.8
AST	92.3 ± 51.2	87.7 ± 47.9	95	95.5 ± 50.6	92.4 ± 48.8	96.7

Table 2: Comparison between group I (I.M) and group II (buccal patches) in significant biochemical changes and their rate of change.

Discussion

Liver cirrhosis is, still nowadays, an important public health problem, mainly in specific geographical areas and among groups with specific socio-economic status. In Egypt in recent years, the prevalence of these infections has decreased [5]. Because of their special bad nutritional characteristics, these patients need a special studies and monitoring. Vitamin B12, hemoglobin, aminotransferase enzymes, and different clinical features were studied to evaluate and develop the patients (volunteers) under the study.

The all vitamin B12 comes from the diet, with vitamin B12 present in all animal foods. After being ingested, vitamin B12 is bound to the intrinsic factor, a protein secreted by the gastric parietal cells. Other cobalamin-binding proteins (called R factors) compete with the intrinsic factor for vitamin B12. Vitamin B12 bound to R factors cannot be absorbed. The vitamin B12- intrinsic factor complex travels through the intestine and is absorbed in the terminal ileum by cells with specific receptors for the complex. It is then transported through plasma and stored in the liver. Three plasma transport proteins have been identified. Transcobalamins I and III (differing only in their carbohydrate structure) are secreted by the white cells. Although approximately 90% of plasma vitamin B12 circulates bound to these proteins, only transcobalamin II is able to transport vitamin B12 into cells. Vitamin B12 is a cofactor for two key metabolic reactions, methylation of homocysteine to methionine and conversion of methylmalonyl coenzyme A to succinyl CoA. It is necessary for tetrahydrofolate production, an important factor for hematopoiesis and DNA synthesis [6].

Vitamin B12 deficit is extremely rare. Information about the prevalence of vitamin B12 deficiency is limited. Normal serum B12 levels range from 200 to 900 pg/mL and levels below 80 are indicative of deficiency, except for inborn errors of metabolism. One of each 200 children has serum levels less than 200 pg/mL [7].

Concerning the demographic features age, sex and BMI were the valuable characters to be studied in patients. The statistical evaluation

of the demographic characters shows no significant differences between the two groups (two different dosage forms) of study. Age is an important factor as it affects the level of malnutrition especially in Cobalamin level [8]. Age range in the two groups of study was from 45-80 (Mean ± SD=60.4 ± 10.8) with p-value=0.951 that ascertain no significant difference between the two groups in age. Age is considers acceptable in this rang as some studies reached to resistance to Cobalamin in elderly and 7-30% of elderly subjects with normal serum Cbl levels have high metabolite values even when serum creatinine is normal [9]. Sex is another factor affected the results as females' needs are more than males in Cobalamin especially during pregnancy and lactation [10]. Males in the study were between 19 (65.5%) and 16 (55.2%) whereas, females were between 10 (34.5%) and 13 (44.8%) with no significant between the two group of study in sex (p-value=0.421). females pregnant or using oral contraceptives were excluded [11]. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in meters. The WHO regards a BMI of less than 18.5 as underweight and may indicate malnutrition, an eating disorder, or other health problems, while a BMI equal to or greater than 25 is considered overweight and above 30 is considered obese [12,13]. These ranges of BMI values are valid only as statistical categories. BMI in the results was between 25.8 ± 4.1 and 26.4 ± 4.6 that means patients were at the first line of overweight. High BMI is associated with type 2 diabetes only in persons with high serum gamma-glutamyl trans peptidase [14]. BMI show no significant difference between the two groups of study (p-value=0.610).

After 3 months of Cobalamin administration data collected show a significant change in biochemical profile of patients in the two groups. In the post-treatment data renal functions don't affected or changed with these supplimation of Cobalamin that may return to that patients chosen with good renal function and there was not any problems in renal clearance so we can say vitamin B12 in doses of 500 μg daily is significant and don't impair renal functions or other biochemical functions in the body [15]. B vitamin therapy is beneficial in patients with good renal function, but harmful in patients with significantly impaired renal function (a glomerular filtration rate <50). It seems likely that in patients with renal impairment, methylcobalamin should be used instead cyanocobalamin [16].

Since the liver plays an important role in the storage and transport of Cobalamin, it is not surprising that liver pathology is associated with major changes in plasma Cobalamin concentrations. For example in acute hepatitis, elevated levels in plasma have been found in 25 to 40% of the patients [17]. Inflammation-induced cell degradation hereby causes the release of stored Cobalamin, which in the circulation predominantly binds to HC. This latter process becomes reinforced by a diminished concentration of TC II, which is the result of an impaired synthesizing capacity of the liver.

In liver cirrhosis the increase of plasma Cobalamin is also associated with tissue depletion. Several studies show a significant decrease of intracellular Cobalamin in liver biopsies [18]. The increase of plasma Cobalamin is related to the severity of the cirrhosis, and can reach 4 to 5 times the upper limit of the reference values. However, hepatocytes are degraded to a lesser degree than is the case in acute hepatitis. It is therefore assumed that a diminished uptake of HC-bound Cobalamin by the affected liver also contributes to the elevated levels of Cobalamin in plasma.

In this study the history of diabetes changed this point of view as patients pretreatment were suffering from vitamin B12 deficiency and need treatment. Add to that, they responded significantly to the treatment. That comes with [19] who find same results in patients with non-alcoholic fatty liver diseases (NAFLD).

The rate of change of vitamin B12 was from 337 to 400% as reported by Yao et al. [20] and Lindenbaum et al. [21] who showed that 40.5% of a healthy elderly group had serum vitamin B12 levels lower than 258 pmol/liter (350 pg/ml) and 15% of those had elevated levels of serum methylmalonic acid. Among elderly patients whose vitamin B12 level were <150 pmol/liter (200 pg/ml), more than 40% had elevated serum methylmalonic acid levels.

The serum vitamin B12 levels were quantified using an ELISA. We defined biochemical vitamin B12 deficiency as serum levels <300 pg/mL [22]. Anemia was defined as Hb, 13 g/dL for males and, 12 g/dL for females based on the WHO guidelines [23]. The blood glucose level was measured using an automated enzymatic method.

Vitamin B-12 contributes to hemoglobin synthesis by activating succinyl CoA, a chemical required to make heme. Succinyl CoA serves as a precursor for heme, and it undergoes several chemical modifications to eventually form an active hemoglobin protein. Without vitamin B-12, you cannot make enough heme to produce functional red blood cells [24]. People with low vitamin B-12 levels develop vitamin B12 deficiency anemia, which is characterized by blood cells poor in hemoglobin. So, it was clear the progress in hemoglobin level post-treatment from 8.2 ± 1.7 to 9.4 ± 1.4 g/dl after Cobalamin administration in the two groups [25].

Hemoglobin levels increased >110% after the treatment with B12. The relationship between Hb and B12 was studied by Rannelli et al. [26]. He presented a case of a previously healthy 55-year-old East African man with severe vitamin B12 deficiency (serum vitamin B (12) 22 pmol/L) secondary to pernicious anemia. He had a severe hypoproliferative megaloblastic anemia with hemolysis (hemoglobin 61 g/L, mean corpuscular volume 99 fL, reticulocytes 0.8%, haptoglobin undetectable), leukopenia (2.7×10^9/L), thrombocytopenia (96×10^9/L), ataxia with central demyelination, and megaloblastic madness. These cases respond to treatment with Cobalamin and recorded increase in levels of hemoglobin. Transferrins [27] and lymphocytes levels had shown significant changes after treatment with Cobalamin. These changes were studied before by Kurnick [28] who suggested that a relative impairment of protein synthesis is common to the decreases in albumin, transferrin, and erythropoietin levels seen in patients with chronic disorders and that this impairment is causally related to their anemias.

Highly significant positive correlations were found between the hepatocellular enzymes AST and ALT as well as vitamin B12. Levels of ALT and AST enzymes show significance changes in their levels in the post-treatment. The relationship between liver enzymes and cobalamin still unclear to us but there are a hypothesis is the most widely accepted model to explain the changes especially in ALT is insulin resistance. Insulin resistance is the key pathogenic factor for the development of hepatic problems with diabetes and highly effect on ALT levels [29,30].

Our alternative explanation is that insulin resistance may be associated with low-level serum vitamin B12 in patients with diabetic cirrhotic patients. Moreover, a current study reported that there may be an association between insulin resistance and low vitamin B12 level [31]. In addition; it was shown that there had been a relationship between insulin resistance and diabetic cirrhotic [32]. The clinical manifestations are highly polymorphic and of varying severity, ranging from milder conditions such as fatigue, common sensory neuropathy,

atrophic glossitis (Hunter's glossitis) and isolated macrocytosis or neutrophil hypersegmentation, to severe disorders, including combined sclerosis of the spinal cord, hemolytic anemia and even pancytopenia [33]. Frequently, neurologic signs and symptoms precede haematologic abnormalities or continue to be isolated. Clinical manifestations of Cobalamin deficiency were clear in the two groups of study.

Gastrointestinal and neurological symptoms and signs were studied and evaluated. Gastrointestinal symptoms as sore tongue, loss of appetite, diarrhea or constipation show significant progression after Cobalamin administration in the two groups of patients.

Vitamin B12 deficiency symptoms and signs include: Gastrointestinal signs and symptoms of vitamin B12 deficiency occur in 26% of cases, as described by Healton et al. [34]. These include sore tongue, stomatitis, mucosal ulceration, appetite loss, flatulence, and constipation or diarrhea. Appetite loss, excess gas, and diarrhea are probably related to the underlying gastric disorder (i.e., gastric atrophy) in pernicious anemia. Gastrointestinal symptoms may occur in the absence of symptomatic anemia or macrocytosis [35]. Others presented some of B12 low levels on GIT as weight loss, diarrhea, vomiting, anorexia, and thickened intestines. Definitive diagnoses in some cases included inflammatory bowel disease (IBD), intestinal lymphoma, cholangiohepatitis or cholangits, and pancreatic inflammation was postulated by Simpson et al. [36].

The development in GIT complains was significant after treatment with B12 in the two groups of study. That reported by many as [8,37,38] who reported the great response after treatment.

Cobalamin deficiency of the nervous system is a progressive disorder, which is manifested by abnormalities of the spinal cord, peripheral nerves, optic nerves, and cerebrum. In 33% of patients, there are sensory disturbances in the extremities (paresthesia or numbness) alone. Motor disturbances alone, especially gait ataxia, are present in 9% of cases. Cognitive impairment may occur, ranging from loss of concentration to memory loss, disorientation, and frank dementia, with or without mood changes. Anosmia, fecal and urinary incontinence, leg weakness, impaired manual dexterity, and impotence are less frequent symptoms. Rare symptoms are orthostatic lightheadedness, diminished taste, paranoid psychosis, and diminished visual acuity [34]. Depending on the duration of symptoms, neurologic complications of vitamin B12 deficiency may or may not be reversible following treatment (the longer the delay before treatment, the less likely recovery). That is returned to Vitamin B12 is necessary for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function. Demyelination of the cervical and thoracic dorsal and lateral columns of the spinal cord, occasional demyelination of cranial and peripheral nerves, and demyelination of white matter in the brain [34] (i.e., "combined-systems disease" or "subacute combined degeneration") can occur with vitamin B12 deficiency.

Diabetic neuropathic symptoms were defined by the presence of typical symptoms, such as pain, burning or aching, prickling sensations, hypoesthesia or numbness in both of the lower legs or feet, through a questionnaire [39]. Patients' response to B12 treatment was significant as reported by many as Bottiglieri [40] who postulate that a defect in methylation processes is central to the biochemical basis of the neuropsychiatry of B12 vitamin deficiencies. And supported with Metz et al. [41].

Conclusion and Recommendation

In this study the history of diabetes changed this point of view as patients pretreatment were suffering from vitamin B12 deficiency and need treatment. Add to that, they responded significantly to the treatment. The descriptive measures of the parameters upon admission were significantly differs after B12 treatment in the two groups of study. After four weeks of treatment; Vitamin B12 showed a positive and significant increase and correlation with all measured parameters. At last, we should point to the difference between two groups who adapted to two different dosage forms of Cobalamin. Group II (buccal patches) show results near to others in Group I (I.M) except in compliance. A patient compliance was significantly high in group II than group I. adds to the progress in B12 levels was so close without pain or the risk of infection.

So, we recommended buccal mucoadhesive films on injection for treatment malnutrition in cirrhotic patients with diabetes. And we recommended more studies on more patients on the new dosage form.

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